FORMULATION AND CHARACTERIZATION OF MAGNETICALLY RESPONSIVE MESALAMINE MICROSPHERES FOR COLON TARGETING

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI-600032.

in partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

Submitted by

Reg. No. 261411261

Under the guidance of

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APRIL 2016

DEPARTMENT OF PHARMACEUTICS
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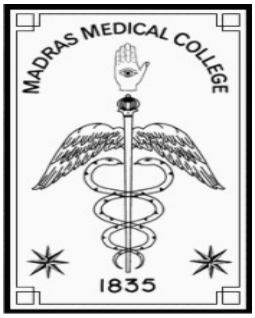

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Place: Chennai – 03
Date:

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Prof. K Elango

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(Dr.R Devi Damayanthi, M. Pharm., Ph.D.)

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LIST OF ABBREVIATIONS AND SYMBOLS

5-ASA	5- Amino Salicylic Acid
BP	British Pharmacopoeia
CTDDS	Colon Targeted Drug Delivery
CD	Crohn's Disease
CFU	Colony Forming Units
DDS	Drug Delivery Systems
DNA	Deoxyribo Nucleic Acid
DSC	Differential Scanning Colorimetry
FDA	Food and Drug Administration
FT-IR	Fourier Transform Infra Red
G	Gauss
GIT	Gastro Intestinal Tract
HCl	Hydrochloric acid
IBD	Inflammatory Bowel Disease
IL	Interleukins
JP	Japanese Pharmacopoeia
MM	Magnetic Microspheres
MRI	Magnetic Resonance Imaging
O/O	Oil in Oil
OROS-CT	Oral Osmotic System for Colon Targeting
Ph. Eur	European Pharmacopoeia
RES	Reticulo Endothelial System
RH	Relative Humidity

SD	Standard Deviation
SEM	Scanning Electron Microscopy
SLS	Sodium Lauryl Sulphate
TDDS	Targeted Delivery System
TNF	Tumor Necrosis Factor
UC	Ulcerative Colitis
UV	Ultraviolet
USP	United States Pharmacopoeia
VSM	Vibrating Sample Magnetometer
emu	Electromagnetic unit
mg	Milligram
g	Gram
ml	Milliliter
µm	Micrometer
% w/w	Percentage weight by weight
min	Minute
mm	Millimeter
Hrs	Hours
µg	Microgram
nm	Nanometer

1.1 NOVEL DRUG DELIVERY SYSTEM

Novel drug delivery research aims to conveniently administer complex drugs to the target tissue in the biological system in a more stable and reproducible controlled way so that it would achieve higher activity at a minimal dose for prolonged period at the site devoid of side effects. Entrapment of a drug into a polymeric system may protect the drug from inactivation and help to retain its activity for prolonged durations, decrease its toxicity, dosing frequency and offers flexibility in administration. A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. [1]

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems, are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology.

To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g., pH- or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest).[2]

1.2 TARGETED DRUG DELIVERY SYSTEM (TDDS)

Targeted drug delivery is defined as a method of delivering medication to a patient in a manner that increases the concentration of the medication in certain parts of the body relative to others. In traditional drug delivery systems like oral ingestion and IV injection, the medication is distributed throughout the body through the systemic blood circulation. For most drugs, only a small portion of the medication reaches the affected organ. Targeted drug delivery system aims at concentrating the medication in the tissues of interest and hence reducing the relative concentration of the medication in the remaining tissues. This helps in reducing the side effects and improving the efficacy.[3]

1.2.1 CHARACTERISTICS OF AN IDEAL TDDS

- Non-toxic, non-immunogenic, physically and chemically stable *in-vivo* and *in-vitro*.
- Should restrict drug distribution to target cells or tissues or organs.
- Should have uniform capillary distribution.
- Controllable rate and therapeutic amount of drug release.
- Drug release should not affect the drug action.
- Drug leakage during transit must be minimal
- Carriers used should be bio-degradable or readily eliminated from the body.
- The preparation should be easy or reasonably simple and cost effective.

Targeted Drug Delivery Systems have been classified as follows:[4]

Classification I

1. Site-directed targeting
2. Site-avoidance targeting

Classification II

Site-directed targeting can further be classified as

1. **Passive targeting:** refers to natural or passive disposition of a drug-carrier based on the physiochemical characteristics of the system in relation to the body.

2. **Active targeting:** refers to alteration of the natural disposition of the drug carrier, directing it to specific cells, tissues or organs. For e.g. use of ligands or monoclonal antibodies which can target specific sites.
3. **Inverse targeting**
4. **Dual targeting**
5. **Double targeting**
6. **Combination targeting**

Classification III

Active targeting can be further classified into three broad categories-

1. **First order targeting:** refers to DDS that delivers the drugs to the capillary bed or to the active site.
2. **Second- order targeting:** refers to DDS that delivers the drug to a specific cell type such as the tumour cells and not to the normal cells.
3. **Third- order targeting:** refers to DDS that delivers the drug to the intracellular site of the target cells.

Classification IV

Active targeting can also be categorised as

1. **Organ targeting**
2. **Cellular targeting**
3. **Sub-cellular targeting**

Classification V

1. Biochemical targeting

2. Biomechanical targeting

3. Biophysical targeting

4. Bioadhesive targeting

Classification VI

1. **Carrier dependent targeting**
2. **Carrier independent targeting**

Drug targeting requires carriers for selective delivery which can:

- ✓ Protect the drug from degradation after administration
- ✓ Improve transport or delivery of drug to cells
- ✓ Decrease clearance of drug
- ✓ Combination of the above

1.2.2 CARRIERS FOR DRUG TARGETING

1. **Carriers covalently bonded to drug:** where the drug release is required for pharmacological activity.

2. **Carriers not covalently bonded to drug:** where simple uncoating of the drug is required for pharmacological activity. Eg. Liposomes.

The various carriers used for drug targeting are-

- Microspheres
- Nanoparticles
- Polymeric carriers
- Albumin
- Lipoprotein
- Liposomes
- Niosomes
- Antibodies
- Cellular carriers and
- Macromolecules.

1.3 COLON TARGETED DRUG DELIVERY SYSTEM (CTDDS)

CTDDS means targeted delivery of drugs into the lower GIT, which occurs primarily in the large intestine (i.e. colon). In the past two decades, the pharmaceutical scientists have extensively investigated in the area of colonic region for targeted drug delivery.[5] Colon targeting depends on exploiting a unique feature of specific site and protecting the drug until it reaches to the site.

Targeted drug delivery to the colon is highly desirable for local treatment of a variety of bowel diseases such as inflammatory bowel disease, amebiosis, colonic cancer and for local treatment of local colonic pathologies, and the systemic delivery of protein and peptide drugs.[6] The advent of slow release technologies increases the chances for a drug to be released in the colon and thus this organ has an important role to play in drug absorption from oral sustained release formulations.[7]

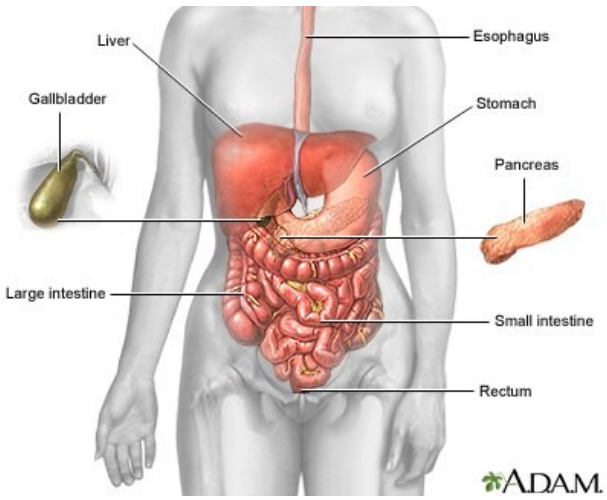


Figure 1: Human Digestive System

1.3.1 Advantages of CTDDS:

- ✓ Reducing the side effects that occur because of release of drugs in the upper GIT or unnecessary systemic absorption
- ✓ The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability.[5]
- ✓ By producing the friendlier environment for peptides and proteins when compared to upper GIT.

- ✓ Preventing the gastric irritation produced by oral administration of NSAIDS.
- ✓ Delayed release of drugs to treat angina, asthma and rheumatoid arthritis.

1.3.2 Limitations of CTDDS:

- ✓ Colon is difficult to access due to its location at the distal portion of the GIT.
- ✓ Successful delivery requires the drug to be in solution before it arrives in the colon, but the fluid content in the colon is lower and more viscous than in upper GIT, which is the limiting factor for poorly soluble drugs.
- ✓ Lower surface area and relative tightness of the tight junctions in the colon can restrict drug transport across the mucosa into the systemic circulation.

1.3.3 Drugs suitable for CTDDS[8]

The different categories of drugs suitable for colon drug delivery are shown in table 1.

Table 1: Colon targeting Diseases, Drugs and Sites

S.No.	Target sites	Disease conditions	Drug and active agents
1.	Topical action	Inflammatory Bowel Disease, Irritable Bowel Disease	Hydrocortisone, Budesonide, Prednisolone, Sulfasalazine, Olsalazine, Mesalazine, Balsalazide
2.	Local action	Chronic Pancreatitis, Fibrosis, colorectal cancer	Digestive enzyme supplements 5-Flourouracil
3.	Systemic action	To prevent gastric irritation	NSAIDS
		To prevent first pass metabolism of orally ingested drugs	Steroids
		Oral delivery of peptides	Insulin
		Oral delivery of vaccines	Typhoid
		Drugs poorly absorbed from GIT	Ibuprofen, Isosorbides

1.3.4 Factors to be considered in the design of colon targeted drug delivery system

Colon act as Black-box of the body and site specificity is difficult task. Various factors are to be considered for designing colon targeted drug delivery.

1.3.4.1 Anatomy and Physiology of colon[8]:

The GIT consists of parts from mouth to anus. It mainly consists of stomach, small intestine and large intestine.

The large intestine extends from the distal end of the ileum (ileocecal junction) to the anus measuring about 1.5meters in length and is divided into three parts- colon, rectum and anal canal. The colon is the upper five feet of the large intestine and mainly situated in the abdomen. It is a cylindrical tube that is lined by mucosa. It includes caecum, colon and rectum. Caecum forms the first part of the colon and leads to the right colon or the ascending colon followed by the transverse colon, the descending colon, sigmoid colon, rectum and the anal canal. The major function of the colon is to create a suitable environment for the growth of colonic microorganisms, absorption of potassium and water from lumen, storage reservoir of faecal contents and expulsion of the contents of the colon at an appropriate time. The retention time of large intestine is 3-10hr.

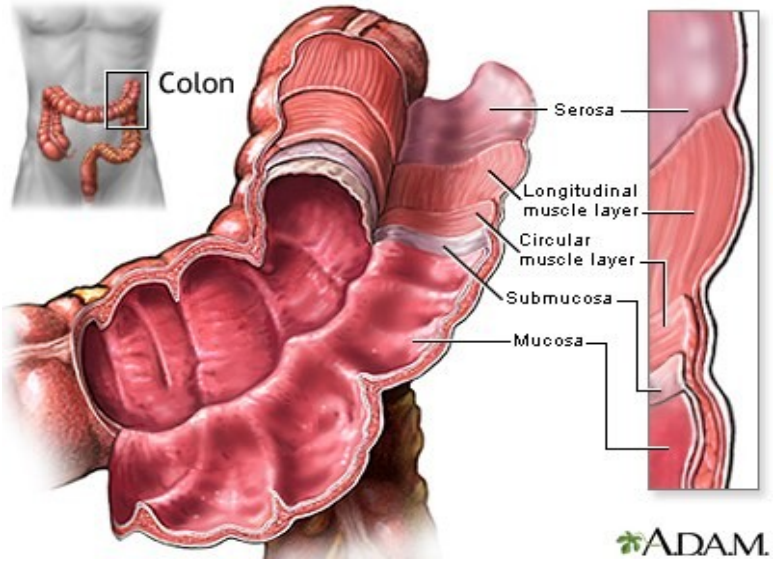


Figure 2: Anatomy of Colon

1.3.4.2 pH of Colon:[8]

The pH of GIT is subject to both inter and intra subject variations due to diet, diseased state, etc. This change in the pH in different parts of GIT is the basis for the development of colon targeted drug delivery systems. Coating with different polymers is done to target the drug to the site.

Table 2: pH in different parts of Colon

Part of GIT	pH
Stomach	Fasted state - 1.5 to 2 Fed state 2 to 6
Small intestine	6.6 to 7.5
Colon Ascending colon Transverse colon Descending colon	 6.4 6.6 7

1.3.4.3 Colonic micro flora & their enzymes:

Drug release in various parts of GIT depends upon the presence of intestinal enzymes. Gut juices and gut microflora. The enzymes are used to degrade coatings/matrices as well as to break bonds between an inert carrier and an active agent (i.e., release of a drug from a prodrug) resulting in the drug release from the formulation. Almost 400 distinct bacterial species have been found, out of which 20% to 30% are of the genus Bactericides. The upper region of GIT consists of very small number of bacteria and predominantly gram-positive facultative bacteria. The concentration of bacteria in the human colon is around 1000 CFU/ml. The most important anaerobic bacteria's are Bactericides. Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, Propionibacterium and clostridium.

1.3.5 COLONIC ABSORPTION OF DRUGS[7]

The surface area of the colon is much less compared to small intestine and is compensated by absence of endogenous digestive enzymes and long residence time of colon (10-24 hours). Routes for colonic absorption are:

- Passes through colonocytes (Trans cellular transport- most lipophilic drugs).
- Passes between adjacent colonocytes (Para cellular transport- most hydrophilic drugs).

Drugs shown to be well absorbed includes Glibenclamide, Diclofenac. Theophylline, Ibuprofen, Metoprolol and Oxyprenolol. Drugs shown to be less absorbed includes Furosemide, pyretanide, Buflomedil, Atenolol, Cimetidine, Hydroclorthiazide, Lithium and Ciprofloxacin.

1.3.6 APPROACHES FOR SITE SPECIFIC DRUG DELIVERY TO COLON[8]

Approaches used for site-specific drug delivery are:

- Primary approaches for CTDDS
  - ✓ pH sensitive polymer coated delivery to colon
  - ✓ delayed (time controlled release system) release drug delivery to colon.
  - ✓ Microbially triggered drug delivery to colon
    - Prodrug approach for drug delivery to colon
    - Azo-polymeric approach for drug delivery to colon
    - Polysaccharide based approach drug delivery to colon
- Newly developed approaches for CTDDS
  - ✓ Pressure controlled drug delivery system
  - ✓ CODES
  - ✓ Osmotic controlled drug delivery to colon (OROS-CT)

1.3.6.1 pH-based systems

Enteric polymers are insoluble in stomach contents and they prevent drug dissolution until the formulation passes into the small intestine. But, they may start to dissolve in the lower small intestine, and hence the site-specificity of formulations becomes poor. The decline in pH from the end of small intestine to the colon can also result in some problems. Lengthy lag times in the ileocecal junction or rapid transit through the ascending colon results in poor site-specificity of enteric-coated single-unit formulations.[8] Asacol®, Claversal®, Mesazal®, Calitoflak®, Entocort® etc. are examples of pH based systems.

1.3.6.2 Delayed (time controlled release system) release drug delivery to colon.

In this approach, the basic principle is the release of the drug after a predetermine lag time from dosage form at the site of action at right time and in right amount.

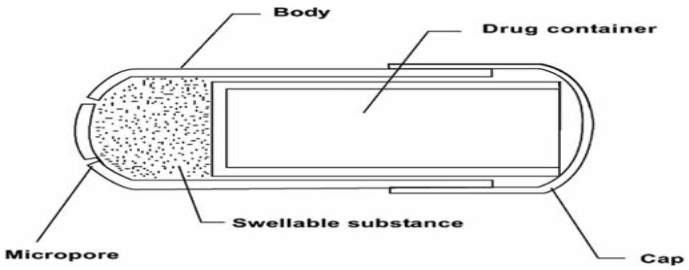


Figure 3: Time controlled and time dependent system

Disadvantages of this system are

(i) Gastric emptying time varies markedly between subjects or in a manner dependent on type and amount of food intake.
Gastrointestinal movement, would result in the change in gastrointestinal transit of the drug.

(ii) Accelerated transit through different regions of the colon has been observed in patients with the IBD, diarrhoea and ulcerative colitis.

1.3.6.3 Microbially triggered drug delivery to colon:

The basic principle involved in this method is degradation of polymers present in the drug delivery systems by microflora present in the colon, thereby release of drug load into colonic region. The colonic bacteria are predominately anaerobic in nature and secrete enzymes that are capable of metabolizing substrates such as carbohydrates and proteins that escape the digestion in the upper GI tract.

The enzymes present in the colon are

1. **Reducing enzymes:**
   - Nitroreductases
   - Azoreductases
   - N-oxide reductases
   - Sulfoxide reductases
   - Hydrogenases
2. **Hydrolytic enzymes:**
   - Esterases
   - Amidases
   - Glycosidases
   - Glucuronidases.

Because of the presence of the biodegradable enzymes only in the colon, the use of biodegradable polymers for colon specific drug delivery seems to be a more site-specific approach as compared to other approaches. These polymers shield the drug from the environments of stomach and small intestine and are able to deliver the drug to the colon. On reaching the colon, they undergo assimilation by micro-organism or degradation by enzyme or break down of the polymer back bone leading to a subsequent reduction in their molecular weight and thereby loss of mechanical strength. They are then unable to hold the drug entity any longer.

Pro drug approach

Pro drug is pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation *in-vivo* to release the active drug. For colonic delivery the pro drug are designed to undergo minimal absorption and hydrolysis in the tracts of upper GIT and undergo enzymatic hydrolysis in the colon, there by releasing the active drug moiety from the drug carrier. It is not very versatile approach as its formulation depends upon the functional group available on the drug moiety for chemical linkage.

Azo-polymeric prodrugs:

Newer approaches are aimed at use of polymers as drug carriers for drug delivery to the colon. Both synthetic as well as naturally occurring polymers are used for this purpose. Sub synthetic polymers have been used to form polymeric pro drug with azo linkage between the polymer and drug moiety. These have been found to be similarly susceptible to cleavage by the azoreductase in the large bowel.

Polysaccharide based delivery systems:

Naturally occurring polysaccharides are found in abundance, widely available, are inexpensive and are available in a variety of structures with varied properties. They can be easily modified chemically and biochemically and are high stable, safe, nontoxic, hydrophilic and gel forming and biodegradable. These include naturally occurring polysaccharides obtained from plants, animals (chitosan, chondroitin), algae (alginates) and microbials (dextran). They are broken down by the colonic microflora to simple saccharides. So these fall into the category of "generally regarded as safe"

1.3.6.4 NEWLY DEVELOPED APPROACHES FOR CTDDS

(i) Pressure-controlled drug-delivery systems (PCDCs)

(ii) Novel colon targeted delivery system (CODES^{TM})

(iii) Osmotic controlled drug delivery (OROS-CT)

Table 3: Marketed Colon- Targeting Dosage Forms

S. No.	Drug	Trade name	Formulation	Presentation	Dose
1.	Mesalamine	Asacol®	Eudragit-S coated tablets (dissolves at pH 7)	400 mg tablets	0.8- 2.4 g/d
2.	Mesalamine	Claversal® Mesazal® Calitoflak®	Eudragit-L coated tablets (dissolves at pH 6)	Tablets	1.0 – 2.0g/d
3.	Mesalamine	Salofac®	Eudragit-L coated tablets (dissolves at pH 6)	400 mg tablets	1.0 – 4.0g/d
4.	Sulfasalzine	Azulfidine	Cellulose acetate phthalate coated tablets	500 mg tablets	2.0 g/d
5.	Sulfasalazine	Colo-Pleon®	Enteric coated tablets	500 mg tablets	2.0g/d
6.	Budesonide	Entocort®	Eudragit-L coated beads (dissolves at pH6)	3 mg capsules	9 mg/d
7.	Budesonide	Budenofalk®	Eudragit-S coated tablets (dissolves at pH7)	3 mg capsules	9 mg/d

1.4 MICROSPHERES

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000µm.

Microspheres are defined as "monolithic spheres or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" or can be defined as structure made up of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level.

There are two types of microspheres:

- Microcapsules
- Micro matrices

Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micro matrices in which entrapped substance is dispersing throughout the microspheres matrix. They are made up of polymeric, waxy, or other protective materials, that is biodegradable synthetic polymers and modified natural products.

1.4.1 TYPES OF MICROSPHERES [14,28]

1. **Bioadhesive microspheres**
2. **Floating microspheres**
3. **Radioactive microspheres**
4. **Magnetic microspheres**[14]

This kind of delivery system is very much important which localizes the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres like chitosan, dextran etc. The different types are therapeutic magnetic microspheres and diagnostic microspheres.

5. **Polymeric microspheres**

5 (a) Biodegradable polymeric microspheres

Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bioadhesive nature. These polymers prolongs the residence time when contact with mucous membrane due to it's high degree of swelling property with aqueous medium, results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. The main drawback is in clinical use drug loading efficiency of biodegradable microspheres is complex is difficult to control the drug release.

5 (b) Synthetic polymeric microspheres

The interest of synthetic polymeric microspheres are widely used in clinical application, moreover that also used as bulking agent, fillers, embolic particles, drug delivery vehicles etc and proved to be safe and biocompatible. The limitation is that these microspheres tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

1.4.2. METHODS OF PREPARATION[28]

1. **Emulsion solvent evaporation technique**
2. **Emulsion-solvent diffusion technique**
3. **Emulsion cross linking method**
4. **Phase separation/ Coacervation**
5. **Spray drying and spray congealing technique**
6. **Multiple emulsion method**

1.4.3 DRUG RELEASE MECHANISM FROM MICROSPHERES[28]

Drug release from the microsphere occurs by general mechanism including diffusion, polymer degradation, and hydrolysis/erosion.

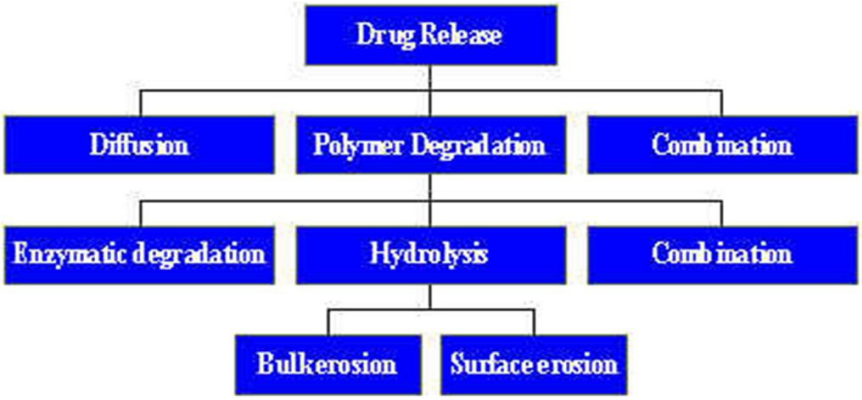


Figure 4: Release mechanism of Microspheres

Diffusion

On contact with aqueous fluids in the gastrointestinal tract (GIT), water diffuses into the interior of the particle. Drug dissolution occurs and the drug solutions diffuse across the release coat to the exterior.

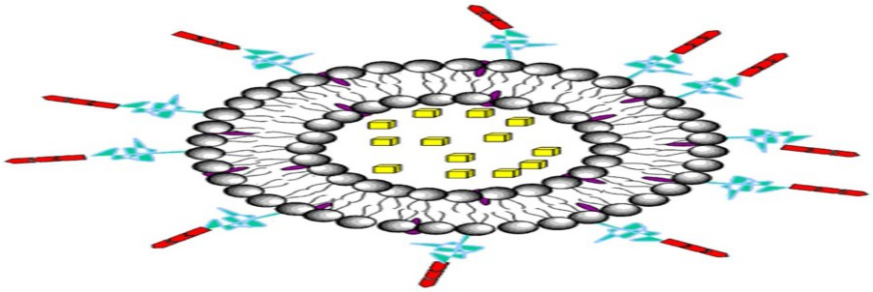


Figure 5: Drug Release from Diffusion

Erosion

Some coatings can be designed to erode gradually with time, thereby releasing the drug contained within the particle. The polymer erosion, i.e. loss of polymer is accompanied by accumulation of the monomer in the release medium. The erosion of the polymer begins with the changes in the microstructure of the carrier as the water penetrates within it leading to the plasticization of the matrix.

1.5 MAGNETIC MICROSPHERES[9]

Drug delivery systems based on microparticles sensitive to a remotely applied magnetic field appear on the top of the (bio) technological innovations, because the magnetic field, if used in a therapeutic level, does not affect biological tissues.[10]

Magnetic drug targeting is a pioneering concept proposed by Freeman et al. in 1960 in which fine iron particle could be transported through the vascular system and concentrated at a particular point in the body with the aid of magnetic fields to achieve prolonged release with high, localized concentrations of drug by retention of the carriers in the region of interest.[32]

Magnetically targeted drug delivery system (MT-DDS) involves binding a drug to a small biocompatible magnetically active component, entrapped in the biodegradable polymer matrix and formulating it into a pharmacologically active stable formulation, which is injected into the blood stream and using a high-gradient magnetic field to pull them out of suspension in the target region.[9]

According to FDA, **Magnetic Microspheres** are supramolecular particles that are small enough to circulate through capillaries without producing embolic occlusion but are sufficiently susceptible (ferromagnetic) to be captured in micro vessels and dragged into the adjacent tissues by magnetic fields of 0.5-0.8 T. Magnetic microspheres (MM) vary widely in quality, sphericity, uniformity, particle size and particle size distribution. The appropriate microspheres need to be chosen for each unique application.[9]

Magnetic microspheres are often formulated with an intention to produce a depot near the target organ, by placing a suitable magnet near it. From the depot, the drug will be released slowly and carried to the target organ through blood. By localizing the drug carrier near the target organ, unwanted distribution of drug to non target organ can be avoided. This approach will localize the drug only at target site and minimize the drug-induced toxicity. The amount and rate of drug delivery via magnetically responsive microspheres can be regulated by varying (i) size of microspheres; (ii) drug content; (iii) magnetite content; (iv) hydration state; (v) drug release characteristic of carrier.[11]

1.5.1 MECHANISMWISE DIFFERENCE BETWEEN MAGNETIC AND NON-MAGNETIC MICROSPHERES[9,14]

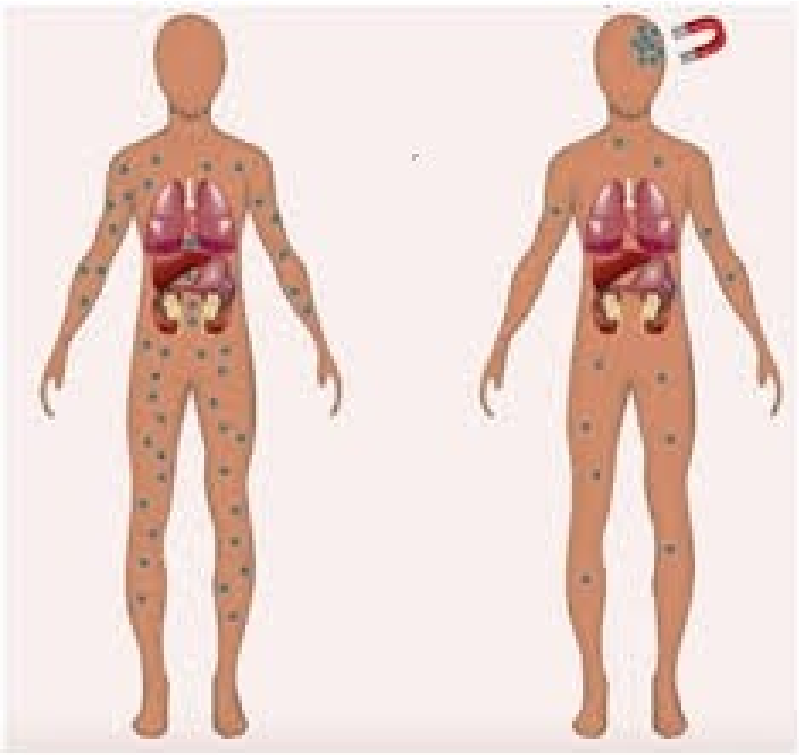


Figure 6: Release mechanism of Microspheres

Various nonmagnetic micro carriers (nanoparticles, microspheres and micro particles etc.) are successfully utilized for drug targeting but they show poor site specificity and are rapidly cleared off by RES (reticuloendothelial system) under normal circumstances. Magnetism plays a vital role in these cases, magnetic carriers such as magnetite, iron, nickel, cobalt, neodymium-iron-boron or samarium-cobalt are used; out of which magnetite being the most common one. It offers the various advantages like (i) Magnetite is well tolerated by the body,(ii) magnetic field is harmless to the body when compared to the traditional radiation methods; (iii) adaptable to any part of the body and, (iv) upto 60% of injected dose can be deposited and released in a controlled manner in selected non-reticuloendothelial organs due to its ability to minimize RES clearance.[11]

1.5.2 CONCEPT AND PRINCIPLE BEHIND MAGNETIC TARGETING [13,14]

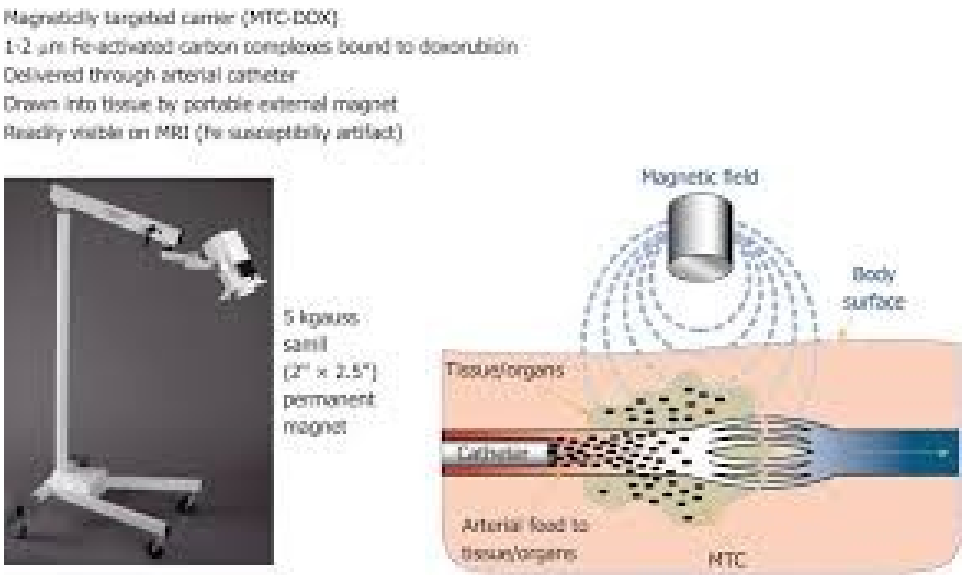


Figure 7: Magnetic Targeting

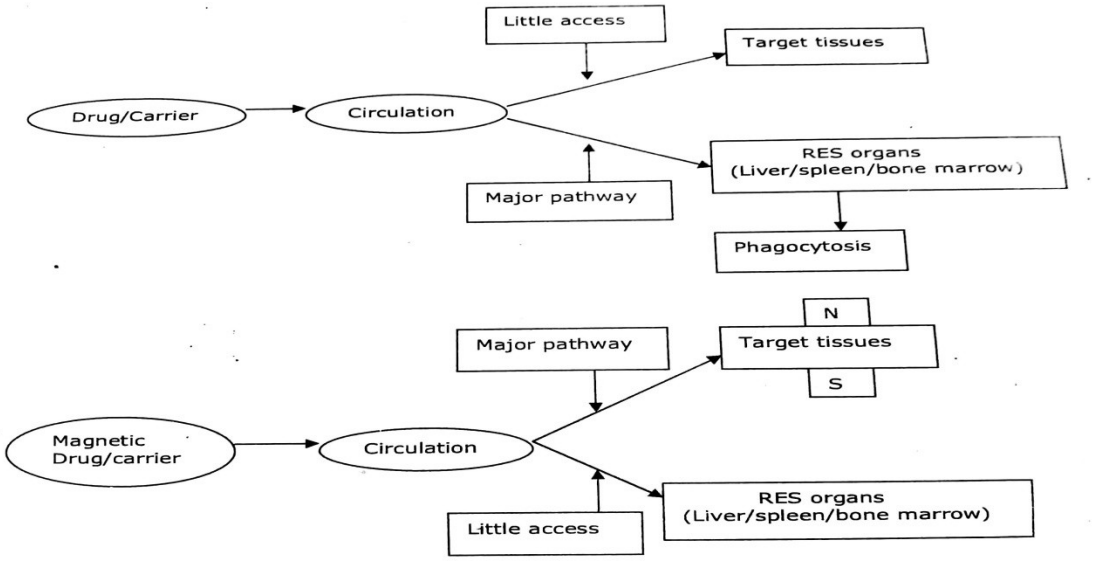


Figure 8: Principle of Magnetic Drug Targeting

Magnetic drug transport is based on the fact that the drug can be either encapsulated into a magnetic microsphere or conjugated on the surface of the microsphere. When loaded MM is introduced into a blood vessel (such as artery) supplying an *in-vivo* target site, accumulation takesplace in the target site to which magnetic field is applied which allows them to deliver the drug locally. Efficiency of accumulation of magnetic carrier on physiological carrier depends on physiological

parameters e.g. particle size, surface characteristics, field strength and blood flow rate etc. Restriction of the microspheres only in the microvasculature can be achieved by taking advantages of the physiological difference in the linear flow velocity of blood in large artery (15-20 cm/sec) versus that in capillaries (0.05cm/sec). The magnetic field helps to extravagate the magnetic microsphere into the target area. Very high concentrations of potent drugs can be achieved near the target site without any toxic effects to normal surrounding tissue or to whole body.[12]

1.5.3 MAGNETIC PROPERTIES [11, 12]

Magnetic particles for bio-seperation consists of one or more magnetic cores with a coating matrix of polymers, silica or hydroxyl apatite with terminal functionalized groups, the magnetic core consists of either magnetite (Fe_3O_4) or magnetite (gamma Fe_3O_4) with superparamagnetic or ferromagnetic properties.

Superparamagnetism is when the dipole moment of a single-domain particle fluctuates rapidly in the core due to the thermal excitation so that there is no magnetic moment for macroscopic time scales. Thus, these particles are non-magnetic in the absence of external magnetic field but do develop a mean magnetic moment in an external magnetic field. Advantages of superparamagnetic particles are easy re suspension, large surface area, slow sedimentation and uniform distribution of the particles in the suspension media. Once magnetized, the particles behave like small permanent magnets, so that they form aggregates or lattice due to magnetic interaction.[11]

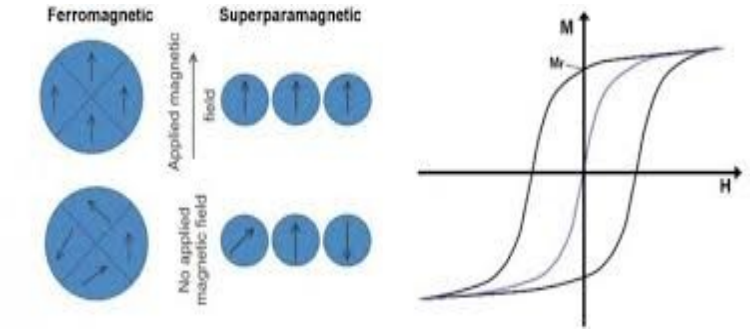


Figure 9: Superparamagnetic particles and Ferromagnetic particles
(a) under the influence of external magnetic field;

(b) in the absence of an external magnetic field

Ferromagnetism means that the particles have a permanent mean magnetic moment. Here, the larger effective magnetic anisotropy suppresses the thermally activated motion of the core moments. Advantages of ferromagnetic particles are very strong magnetic properties and therefore the fast separation with an external magnetic field even in viscous media.[11]

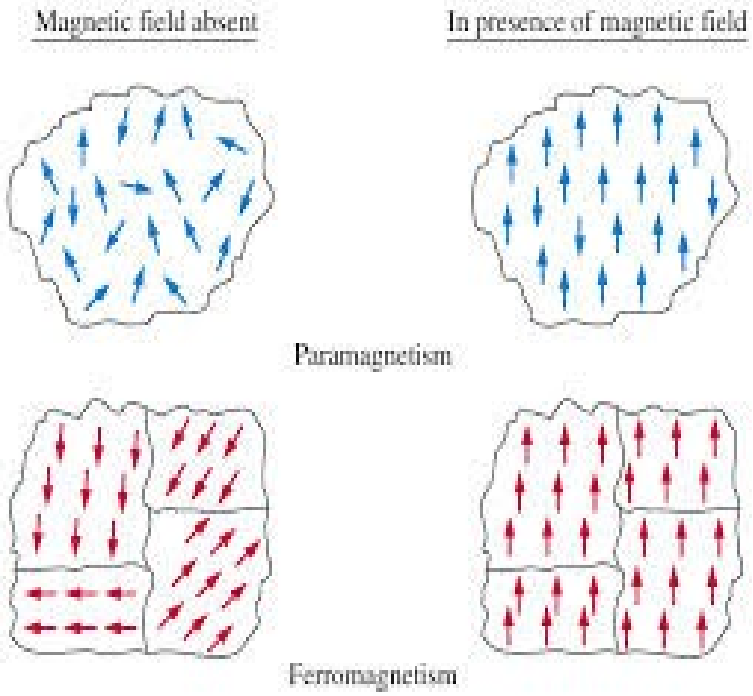


Figure 10: Alignment in Superparamagnetic particles and Ferromagnetic particles
(a) under the influence of external magnetic field;
(b) in the absence of an external magnetic field

1.5.4 MAGNETITE[12]

Also called as ferric ferrous oxide, ferrous ferrite, tri iron tetra oxide, and black iron oxide. Magnetic iron oxide chemical formula $FeO .Fe_2O_3$ having a molecular weight of 231.55 with a chemical composition of Fe = 72.36%, O = 27.64%. The ferromagnetic material when incorporated into microspheres makes them magnetically responsive, so that they can be concentrated to the desired site by applying some external magnetic field.

While employing magnetic particles in drug delivery, an ideal drug carrier should be:

- Small enough to remain in circulation after injection.
- Biocompatible i.e., non-toxic and non-immunogenic.
- Able to cross the anatomic barriers.
- Recognized only by the target cells.
- Not releasing the drug before reaching the target.

1.5.4 MAGNET DESIGN[12]

Magnetic targeting results from the force exerted by a gradient magnetic field. The relationship of magnetic force to field gradient and magnetic moment of particles is expressed by the following equation:

Magnetic force = Magnetic moment x Magnetic field gradient

It is clear from the equation that increased magnetic moments offer forces sufficient for the extravascular migration at proportionately lower field gradients. The magnetic moments of the microspheres can be increased in following three ways:

a) By magnetizing the sphere to saturation level prior to vascular targeting.
b) By clustering magnetite at the centre of each sphere to produce larger macrodomains.
c) By substituting one of the newer ferromagnetic materials that has higher susceptibility than magnetite.[12]

In magnetic microspheres with high magnetic content, the external magnetic field strength required is less, but if high magnetic content is present, then the space available for drug is less and hence the magnitude of magnetic content and drug should be delicately balanced to have an efficient therapeutic system. Satisfactory targeting of microspheres (with 20% magnetite) could be achieved using magnets of 0.55 to 0.80 T field straight. 0.01 T T/mm field gradient and 0.4 to 0.8 cm (diameter).[12]

1.5.5 FACTORS AFFECTING THE PROPERTIES OF MAGNETIC MICROSPHERES[9,13]

1. **Polymer selection**

Materials Used	Types	Examples
Synthetic Polymers	• Non-biodegradable polymers • Biodegradable polymers	PMMA, Acrolein, Glycidyl methacrylate Epoxy polymers, lactides, glycolides and their co-polymers, polyanhydrides
Natural polymers	• Proteins • Carbohydrates • Chemically modified carbohydrates	Albumin, Gelatin, Collagen Agarose, Chitosan, Carageenan, Starch Poly dextran, Poly starch

Table 4: List of polymers used for the preparation of MM

2. **Choice of solvent**

- Should be able to dissolve the chosen polymer
- Poorly soluble in continuous phase
- High volatility and a low boiling point
- Low toxicity
- Alternative components (dispersed phase)

3. **Co-solvent**: organic solvents miscible with water such as methanol and ethanol.
4. **Porosity generator**: increases degradation rate of polymer and improves drug release rate. e.g. sephadex (cross-linked dextran gel).

5. **Continuous phase**:
   a) **Surfactant** :
   - Reduces surface tension of continuous phase.
   - Avoids coalescence and agglomeration of drops.
   - Stabilizes emulsion.

   Various types are

Surfactant type	**Examples**
Non ionic	partially hydrolysed PVA, methyl cellulose, Tween, Span
Anionic	sodium dodecyl sulphate, SLS
Cationic	tri methyl ammonium bromide

Table 5: List of surfactants used for the preparation of MM

   b) **Alternative components**:
   - Antifoaming agents
   - Anti-agglomerating agents.

1.5.7 PREPARATION OF MAGNETIC MICROSPHERES [13]

1.5.7.1 Selection of Drugs

In the selection of a drug for the formulation of MM, following points are to be considered:

- The drug is so dangerous or labile that we cannot allow it to circulate freely in the blood stream.
- The agent is so expensive, that we cannot afford to waste 99.9% of it.
- Requires a selective, regional effect to meet localized therapeutic objective.
- Requires an alternative formulation essential to continue treatment in patients whose systemic therapy must be temporarily discontinued due to life threatening toxicity directed at selective organs.
- Examples of drugs used: Adriamycin, Amphotericin B, Membrane active agents, Prostaglandins, Immuno suppressants etc.

1.5.7.2 Methods of Preparation

1. Phase Separation Emulsion Polymerization[9]

Phase Separation method is specially designed for preparing the reservoir type of the system to encapsulate water soluble drugs. E.g., peptide, proteins.

In matrix type device, the drug is soluble in the polymer phase. Hence, certain preparations are of matrix type particularly, when the drug is hydrophobic in nature. E.g.,steroids.

This process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and addition of an incompatible polymer/ non-solvent/salt / change in pH to the system (magnetic fluid) which makes first polymer to phase separate and engulf the drug particles.

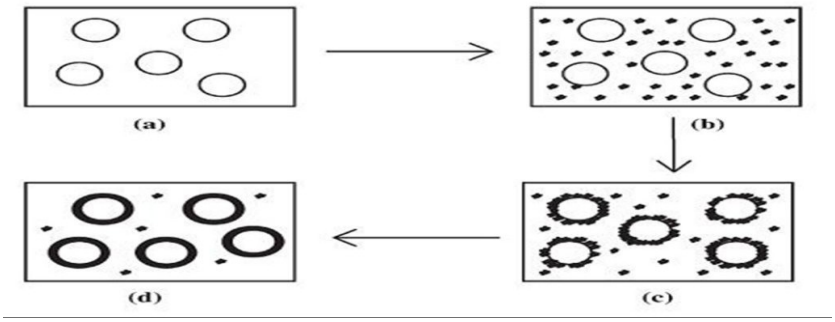


Figure 11: Coacervation process

2. Continuous Solvent Evaporation[11]

In this method the drug and polymer (carrier) are dissolved in appropriate volatile organic solvent and then magnetite is added to this solution along with stirring in order to form a homogeneous suspension. This suspension is added to an immiscible auxillary solution along with vigerous stirring. The volatile organic solvent is evaporated slowly at 22- 30^0 C to form microspheres. Microspheres are then centrifuged and freeze dried and stored.

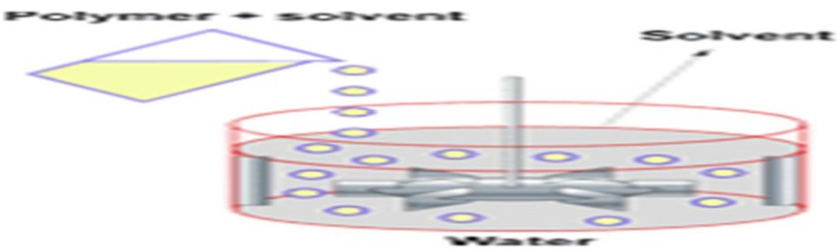


Figure 12: Solvent evaporation technique

3. Ionic Gelation Method[7]

Inotropic gelation is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydrogel beads also called as microspheres. Microspheres are spherical cross-linked hydrophilic polymeric entity capable of extensive gelation and swelling in simulated biological fluids and the release of drug through it controlled by polymer relaxation. The hydrogel beads are produced by dropping a drug loaded polymeric solution into the aqueous solution of polyvalent

cations. The cations diffuses into the drug loaded polymeric drops, forming a three dimensional lattice of ionically cross-linked moiety.

4. Solvent Extraction Method[7]

This method involves removal of organic phase by extraction of the organic solvent. Isopropanol can be used as water miscible organic solvent. By extraction with water, organic phase is removed. Hardening time of microspheres can be decreased by this method.

5. Spray drying and spray congealing technique[35]

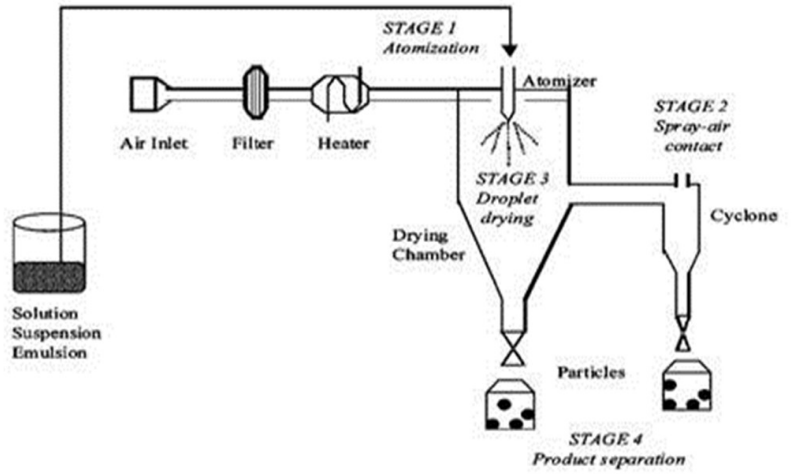


Figure 13: Spray drying technique

These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which

the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100 μm. Microparticles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying. One of the major advantages of the process is

1. Feasibility of operation under aseptic conditions.
2. The spray drying process is used to encapsulate various penicillins. Thiamine mononitrate14 and sulpha ethylthiadizole15 are encapsulated in a mixture of mono- and diglycerides of stearic acid and palmitic acid using spray congealing.
3. Very rapid solvent evaporation, however leads to the formation of porous microparticles.

6 Multiple emulsion method[11]

Multiple emulsion method involves formation of (o/w) primary emulsion (non aqueous drug solution) and then addition of primary emulsion to external oily phase to form o/w/o emulsion followed by either addition of cross linking agent (glutaraldehyde) and evaporation of organic solvent. Multiple emulsion method of preparation is ideal for incorporating poorly aqueous soluble drug, thus enhancing its bioavailability.

7 Emulsion cross linking method[17]

In this method drug was dissolved in aqueous gelatin solution which was previously heated for 1hr at 40°C. The solution was added drop wise to liquid paraffin while stirring the mixture at 1500 rpm for 10min at 35°C, results in W/O emulsion then further stirring is done for 10min at 15°C. Thus the produced microspheres were washed respectively three times with acetone and isopropyl alcohol which then air dried and dispersed in 5ml of aqueous glutaraldehyde saturated toluene solution at room temperature for 3hrs for cross linking and then was treated with 100ml of 10mm glycerine solution containing 0.1% w/v of tween 80 at 37°C for 10min to block unreacted glutaraldehyde.

1.5.8 PHYSICOCHEMICAL CHARACTERIZATION AND EVALUATION METHODS [5,9]

1. Particle size and shape: Laser microscopy, Scanning electron microscopy
2. Electron spectroscopy for chemical analysis: Determines surface chemistry
3. Attenuated total reflectance Fourier Transform-Infrared Spectroscopy: Determines degradation of polymeric matrix
4. Capture efficiency: % Entrapment : UV spectroscopy
5. Thermal analysis: Differential Scanning Calorimetry (DSC)
6. Swelling index: Characterization of microspheres is performed with swelling index technique.
7. Micromeritic properties such as tapped density, bulk density, compressibility index, angle of repose are also studied.
8. Release studies: For different type of microspheres are carried out by using different suitable dissolution media with and without enzymes.
9. Magnetic characterization studies: Vibrating Sample Magnetometer (VSM), percentage magnetite content determination.

1.5.9 ADVANTAGES OF MAGNETIC MICROSPHERES[9]

1. Avoidance of first pass effect: by minimizing RES clearance.
2. Increased duration of action: since MM can transit into extra vascular space creating an extra vascular depot of drugs for sustained release of drugs within the targeted areas.
3. Controlled and predictable rate of drug release with smaller doses of drug can be achieved. Therapeutic response in target organs at only one tenth of free drug dose is achieved.
4. Reduced side effects and reduced toxicity.
5. Method of preparation is simple.
6. Ability to bind and release higher concentration of drugs.
7. It can be injected into the body using hypodermic needle.
8. In case of tumour targeting, microspheres can be internalized by tumour cells due to its much higher phagocytic activity as compared to normal cells.

9. Problem of drug resistance due to inability of drugs to be transported across the cell membrane may be surmounted.
10. Avoidance of acute toxicity directed against endothelium and normal parenchyma cell, controlled release within target tissue for intervals of 30 min to 30 h as desired, adaptable to any part of the body.

1.5.10 DISADVANTAGES OF MAGNETIC MICROSPHERES[11]

1. The drug cannot be targeted to deep-seated organs in the body.
2. Thrombosis at the site of catheterization.
3. Unknown toxicity of magnetic beads.

1.5.11 APPLICATIONS[7,9]

1.5.11.1 Clinical Applications

- It is used as a chemotherapeutic agent. (Loco regional cancer treatment).
- Magnetic vehicles are used for delivery of therapeutic agent as they can be targeted to specific location in the body through the application of magnetic field.
- Magnetic microspheres can be used for stem cell extraction and bone marrow purging.
- Magnetic fluid purging. (Presentation of a new magnetic field therapy system for the treatment of human solid tumors)

1.5.11.2 Biological Applications[14]

- Used in enzyme immobilization-free enzymes are immobilized onto magnetic carriers' surface or porous wall by taking advantage of physical adsorption or covalence. Compared with other immobilized carriers, magnetic carriers have the following advantages:

- ✓ Immobilized enzymes are easily separated from the products or reactants.
- ✓ The manner of movement of immobilized enzymes can be controlled by external magnetic fields, which can greatly enhance the catalyzing efficiency of enzyme.
- ✓ Enzyme catalytic reactions can be continuously carried out and controlled by a magnetic field in a bioreactor, which can reduce the consumption of enzyme.
- ✓ Reutilization of enzyme will reduce the cost.
- ✓ Used for cell isolation, protein purification and targeted drugs.

- Drug discovery, molecular targeting and undergoing the pathway of cell cycle regulation.
- High throughput DNA isolation. (Magnetic bead purification of labelled DNA fragments for high throughput capillary electrophoresis sequencing), DNA analysis and proteomics.
- Magnetofection: a methodology based on the association of magnetic nanoparticles with gene vectors inorder to enhance gene transfer in the presence of a magnetic field.

1.5.11.2 Other Applications

- Non targeted applications of magnetic microparticles include their use as contrast agent (MRI).
- Used as drug reservoirs and in drug transport
- In hyperthermia.
- In radionuclide delivery.

1. **Satinder Kaker *et al.*[14]** prepared magnetic microspheres of mesalamine by phase separation emulsion polymerisation technique for colon targeting using Eudragit S100, Ethyl cellulose and Chitosan polymers. Entrapment efficiency and magnetite content by titrimetry was performed. From the *in-vitro* release study it was observed that with increase in drug polymer ratio, particle size increases, thus surface area is decreased and release of drug is retarded. Chitosan magnetic microspheres prepared by phase separation emulsion polymerisation are found to be the best in all evaluation parameters (practical yield, magnetite content, magnetic responsivity, particle size and *in-vitro* release study.

2. **Seema Badhana *et al.*[39]** prepared colon specific drug delivery of Mesalamine using Eudragit S 100- coated chitosan microspheres for the treatment of ulcerative colitis by ionic gelation emulsification method using Tripolyphosphate as cross linking agent. The microspheres were coated with Eudragit S 100 by the solvent evaporation technique. The prepared microspheres were evaluated and the drug release of Mesalamine from microspheres was found to decrease as the polymer concentration increases. It was observed that Eudragit S 100 coated chitosan microspheres gave no release in the simulated gastric fluid, negligible release in the simulated intestinal fluid and maximum release in the colonic environment.

3. **Ahmed Abd El-Bary *et al.*[16]** investigated some formulation variables on the optimization of pH dependent, colon-targeted, Sustained-release Mesalamine Microspheres. The microspheres prepared by O/O emulsion solvent evaporation method, employing Eudragit S and hydrophobic pH-independent Ethyl cellulose ppolymers. Formulation variables studied included concentration of Eudragit S in the internal phase and the ratios between: internal to external phase, drug to Eudragit S and Eudragit S to Ethyl cellulose to Mesalamine. Particle size and encapsulation efficiency increased by increasing Eudragit S concentration in the internal phase, ratio of internal to external phase, and ratio of Eudragit S to the drug. The results was showed that microencapsulation of Mesalamine in microspheres using blend of Eudragit S and Ethyl cellulose could constitute a promising approach for site-specific and controlled delivery of drug in colon.

4. **Satinder Kakar *et al.*[13]** prepared and evaluated magnetic microspheres of mesalamine for colon drug delivery. Magnetic microspheres were prepared by solvent evaporation technique in the drug polymer ratios 1:1, 1:2, and 1:3 using chitosan as the polymer. The microspheres ere characterised in terms of particle size, percentage yield, drug content, encapsulation efficiency, *in-vitro* release pattern and ex-vivo study.

5. **Murat Turkoglu *et al.*[114]** developed pectin–HPMC compression coated 5-aminosalicylic acid tablets for colonic delivery. They Prepared 5-ASA core tablets by wet granulation method using PVP (K 29-32). Each 100 mg core tablet contained 5-ASA and was compression coated at 20 kN or 30 kN using 100% pectin, 80% pectin–20% HPMC and 60% pectin–40% HPMC at two different coat weights as 400 or 500 mg. Drug dissolution/system erosion/degradation studies were carried out in pH 1.2 and 6.8 buffers using a pectinolytic enzyme. HPMC addition was required to control the solubility of pectin. The optimum HPMC concentration was 20% and such system would protect the cores up to 6 h that corresponded to 25–35% erosion and after that under the influence of pectinase the system would degrade faster and delivering 5-ASA to the colon.

6. **Josephine leno jenita .J *et al.*[100]** Formulated and evaluated the compression coated tablets of Mesalazine for colon delivery. In this study, natural polysaccharide; Locust bean gum (550,450,350 and 250 mg) are used. The *in-vitro* release studies carried out in 0.1N HCl, Phosphate buffer pH 7.4 and Phosphate buffered saline pH-6.8 containing 4% w/v of rat caecal contents. These studies proved the Locust bean gum can able to protect the core tablet containing Mesalazine under conditions mimicking mouth to colon transit and clearly established that Locust bean gum in the form of compression coat is a potential carrier for colon targeting.

7. **Satinder Kakar *et al.*[38]** formulated and evaluated 6-Thioguanine loaded magnetic microspheres by continuous solvent evaporation technique inorder to target the magnetic microspheres to the cancerous site. The prepared microspheres were characterised in terms of percentage practical yield, micromeritic properties, particle size, swelling kinetics, magnetic responsiveness, magnetite content and *in-vivo* drug

release study. Swelling kinetics of magnetic microspheres were performed and it was found that their swelling rate increased with time. Magnetic content was determined by titrimetric method using thiosulphate and potassium iodide for quantitative analysis.

8. **Vyas M.B *et al.*[28]** designed and characterised Cisplatin magnetic microspheres by phase separation emulsion polymerisation technique using bovine serum albumin. Magnetite was prepared by reacting 10%w/v ferrous sulphate with 20%w/v sodium hydroxide solution, followed by washing of the precipitate with dilute ammonia. Percentage magnetite content was determined by conventional titrimetric method using thiosulphate and potassium iodide for quantitative analysis. It was observed that entrapment of magnetite increased with increase in concentration of polymer. The maximum magnetite content was found to be 55.48%. In-vitro drug release studies shows that the percentage drug release decreased with increase in ratio of polymer added. The study is aimed at the overall improvement in the efficacy, reduction in toxicity and enhancement of therapeutic index of cisplatin.

9. **Liang Guo *et al.*[29]** prepared Chitosan Poly(acrylic acid) magnetic microspheres by encapsulating dextran coated Fe_3O_4 nanoparticles into chitosan poly(acrylic acid) (PAA) microspheres template and characterized by Transmission Electron Microscopy(TEM), Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), X-ray powder diffraction (XRD) and Thermogravimetry (TG). Water-dispersible superparamagnetic Fe_3O_4 magnetic nanoparticles with 20-30nm were prepared by precipitation method in aqueous solution of dextran. The chitosan-PAA microspheres were then prepared by polymerizing acrylic acid into chitosan template in aqueous solution followed by encapsulation of dextran coated Fe_3O_4 nanoparticles into it by cross-linking with gluteraldehyde. Magnetic properties were evaluated from the magnetization curves obtained by vibrating sample magnetometer(VSM) and was found that when the magnetic component size of the particles is smaller than critical size, the particles will exhibit superparamagnetism. The results showed that the formed microspheres demonstrated magnetic behaviour in an applied magnetic field. In addition, the magnetite particles were well encapsulated

and the composite particles having high magnetite content, which was more than 40%.

10. **Maria Christina Mascolo *et al.*[79]** synthesised magnetite nanoparticles using co-precipitaion reaction at room temperature in the presence of different bases such as NaOH, KOH or $(C_2H_5)_4NOH$. Magnetite shows characteristics of superparamagnetism at room temperature and a saturation magnetization (Ms) value depending on both the crystal size and the degree of agglomeration of individual nanoparticles. The magnetization curve for the synthesised nanoparticles does not show any hysteresis behaviour which confirmed that the synthesized particles exhibit superparamagnetic properties at room temperature and was found that the Ms value decreases as the magnetite particle size decreases.

11. **Ling-Han Xiao *et al.*[42]** synthesised magnetic-antimicrobial-fluorescent multifunctional hybrid microspheres with well-defined nanostructure by the aid of a poly(glycidyl methacrylate) (PGMA) template. The magnetic hysteresis loop of the M-PGMA/PHGH-CdTe hybrid microspheres displayed a super-paramagnetic property, with a saturation magnetization value 4.608emu/g which is sufficient for magnetic separation from water solution. This Ms value is less than that of the pure magnetic iron oxide nanoparticles (61.87 emu/g), which can be explained by the diamagnetic contribution of PGMA template surrounding the magnetic nanoparticles. After poly (hexamethylene guanidine hydrochloride) (PHGH) functionalization, the resultant microspheres exhibit excellent antibacterial performance. The fluorescent feature originating from the quantum dot CdTe endowed the hybrid microspheres with targeted localization and biological monitoring functions.

12. **Elisangela P. Da Silva *et al.*[43]** developed covalent TiO_2-co-pectin microspheres containing Fe_3O_4 nanoparticles through an ultrasound-induced crosslinking/ polymerization reaction between the glycidyl methacrylate from vinyl groups in TiO_2 and in pectin. The nanostructured pectin microspheres showed an amoxicillin release rate slower than that of pure pectin microspheres. The focus of the work was to develop a smart biodevice based on magnetic pectin microspheres that shows a sustained release profile and further modulated by exposition to a remote magnetic field. The cytotoxic concentrations for 50% of VERO cells (CC_{50}) calculated as the

concentration required to reduce cell viability by 50% after 72hrs of incubation for pectin only microspheres and nanostructured pectin microspheres were 217.7± 6.5 and 121.5± 4.9μgmL^{-1} which showed that the pectin microspheres have great pharmacological potential for use in biological environments even after the introduction of both Fe_3O_4 and TiO_2.

13. **Prabhu Chakkarapani *et al.*[32]** developed methotrexate magnetic microcapsules (MMC) for targeted rheumatoid arthritis therapy. Methotrexate was loaded into $CaCO_3$-PSS (poly(sodium 4-styrenesulfonate)) doped microparticles that were coated by layer-by-layer technique. Ferrofluid was incorporated between the polyelectrolyte layers. The MMC were evaluated and was found that spherical particles of approx. 3μm size were obtained with a net zeta potential of +24.5 mV, 56% encapsulation and 18.6% drug loading capacity, 96% of cumulative drug release obeyed Hixson-Crowell model release kinetics. DC magnetization analysis carried out using the vibrating sample magnetometer (VSM) technique confirmed the superparamagnetic behaviour of the prepared microcapsules.

14. In the website:***www.shodhganga.inflibnet.ac.in***[78] it had been stated that microspheres containing magnetite of 20-28% w/w could be effectively targeted at the site by using magnet of 5000- 8000 Gauss (Guptha and Hung, 1989). The magnetite content of prepared gelatine magnetic microspheres was between 27-29% w/w and was sufficient to retain the microspheres at the site of targeting by using a magnet of 8000 Gauss. It had been proposed that less than 5μm size is used for intravenous route, less than 125 μm is used for intra-arterial route and intra-articular route. Particles of this size can be administered easily by suspending them in a suitable vehicle and injecting them using a conventional syringe with an 18 or 20 gauge needle. The average particle size of gelatin microspheres without drug and magnetite was 15.3 μm. Incorporation of magnetite to this microspheres increased the average size to 22.5 μm. The average particle size of gelatin magnetic microspheres was slightly increased by drug loading to around 25-30.6 μm.

15. **Naveen K Thakral *et al.*[25]** prepared Valdecoxib loaded multiparticulate system to achieve site-specific drug delivery to colorectal tumors. Film coating was done with the pH-sensitive polymer Eudragit S 100 and sodium alginate was used as

mucoadhesive polymer in the core. When applied to the mucosal surface of freshly excised goat colon, microspheres pretreated with phosphate buffer pH 7.4 for 30 minutes showed mucoadhesion. The microspheres were found to exhibit slower and delayed release and lower intracellular concentration of Valdecoxib.

16. **Amol Paharia *et al.*[60]** prepared Eudragit coated Pectin microspheres for colon targeting of 5-Fluorouracil. Pectin microspheres were prepared by emulsion dehydration method using different ratios of drug and Pectin (1:3 to 1:6). Eudragit-coating of pectin microspheres was performed by oil in oil solvent evaporation method using coat:core ratio (5:1) the release profile of Fluorouracil from Eudragit-coated pectin microspheres was pH dependent. In acidic medium, the release rate was much slower; however, the drug was released quickly at pH 7.4. Eudragit-coated pectin microspheres are promising controlled release carriers for colon targeted delivery of Fluorouracil.

17. **Sandeep Kumar *et al.*[22]** prepared and evaluate colon specific microspheres of Meloxicam. The microspheres were prepared by iontropic gelation method using different ratios of Meloxicam and sodium alginate. Eudragit coating of Meloxicam sodium alginate microspheres was performed by coacervation phase separation technique. The microspheres were characterized by shape, particle size, size distribution, incorporation efficiency, *in-vitro* drug release and stability studies. It is concluded from the present investigation that Eudragit coated sodium alginate microspheres are promising controlled release carriers for colon-targeted delivery of Meloxicam.

18. **Josephine Leno Jenita J *et al.*[58]** prepared pH sensitive polymer coated micro particles containing Curcumin for colon targeting by solvent evaporation technique. Eudragit S 100 was selected as a model encapsulation material. The micro particles prepared were filled in hard gelatin capsules which were enteric coated with cellulose acetate phthalate (CAP) which prevent the burst effect of capsule in acidic pH of stomach. The *in-vitro* release study showed that the drug release was between 66.12%-71-87% for 10 hours and results indicate that release of drug from microspheres followed Fickian diffusion mechanism.

19. **Kishori L *et al.*[18]** formulated Tinidazole microspheres for colon targeted drug delivery system by emulsion solvent evaporation method by using Eudragit polymer. A 3^2 factorial design was employed in formulating the microspheres with concentration of surfactant (A) and stirring speed (B) as independent variables. The particle size and entrapment efficiency were found to be varied by changing various formulations parameters like surfactant concentration and stirring speed etc. The release profile of Tinidazole from eudragit microspheres was pH dependent. In acidic medium, the release rate was much slower, however, the drug was released quickly at pH 7.4. It was concluded that eudragit based Tinidazole microspheres offer a high degree of protection from premature drug release in simulated upper GIT conditions and deliver most of the drug load in the colon and allow drug release to occur.

20. **Behin Sundara Raj *et al.*[12]** formulated eudragit coated chitosan microspheres of 5-fluorouracil for colon targeting. Chitosan microspheres were prepared by emulsion-dehydration method using different rations of Fluorouracil and Chitosan (1:2 to 1:6), stirring speed (500-2000rpm) and emulsifier concentrations (0.75% to 1.75% w/v). The results showed that the amount of the polymer increased the extent of drug release decreased.

21. **Dinesh Chandra *et al.*[44]** developed multiparticulate system of Eudragit based Satranidazole microspheres exploiting pH sensitivity property and specific biodegradability for colon targeting. Eudragit S 100 based microspheres were prepared by oil-in-oil solvent evaporation method using different drug-polymer ratios (1:1 to 1:5), stirring speeds (1400 rpm) and emulsifier concentrations (0.5%-1.5%w/v). All formulations were evaluated and release profile of Satranidazole from Eudragit microspheres was pH dependent. In acidic medium, the release rate was much slower; however the drug was released quickly at pH 7.4. The release kinetics revealed that drug followed zero order kinetics followed by Higuchi and first order model.

22. **Rashmi Sareen *et al.*[93]** fabricated and characterized solid lipid based microspheres (SLM) of Ketoprofen(KPF) by hot melt microencapsulation technique and compared its anti-inflammatory potential with the marketed formulation. Results revealed that Tween80 resulted in exceptional KPF entrapment efficiency of 82.6% with spherical

rough surface morphology. The *in-vitro* drug release showed intial burst release of 47% upto 2h followed by sustained release of 70% for 12h and was observed that the formulations obeyed Higuchi model and Korsmeyer-Peppas model of release kinetics. Further in-vitro anti-inflammatory activity by inhibition of albumin denaturation. Briefly 1ml sample solution was withdrawn during in-vitro drug release study and subjected to anti-inflammatory analysis by adding 1ml of bovine albumin and incubated for 30min at 37^0C after pH adjustment to 6.3 followed by further incubation at 57^0C for 5min. After cooling the turbidity of samples were recorded spectrometrically at 660nm. To conclude, SLMs were found to posses superior *in-vitro* and *in-vivo* anti-inflammatory potentials when compared to marketed formulation.

23. **P. Padmanabhan *et al.*[95]** formulated a herbal preparation (HP-4 of 100mg/10ml methanol concentration) using 80% alcoholic extract of leaves of Aloe Vera, Bacopa monnieri, Moringa oleifera and rhizome of Zingiber officinale. Different concentrations of HP-4 was used to study the in-vitro anti-inflammatory activities in terms of effect of hypotonic solution-induced haemolysis on RBC membrane stabilization and effect of inhibition of protein denaturation activity using Acetyl salicylic acid as the reference drug. In their study, they have stated that "several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. (**Grant *et al.,* *1970).*** Denaturation of proteins is a well documented cause of inflammation in conditions like rheumatoid arthritis (**Mizushima *et al.,*** 1964). Thus protection against protein denaturation, which was the main mechanism of action of NSAIDs postulated by (**Mizushima *et al.,*** 1964) before the discovery of their inhibitory effect on cyclooxygenase by **Vane JR**, 1971 played an important role in the antirheumatic activity of NSAIDs." It was found that HP-4 has dose dependent RBC membrane stabilization and inhibition of protein denaturation activity.

24. **Ramana G and Krishna Chaitanya A[76]** prepared and characterised Ethyl cellulose coated pectin alginate microspheres of 5-Flurouracil for colon targeting. Pectin microspheres were prepared by ionotropic external gelation technique and the drug loaded pectin microspheres were coated with Ethyl cellulose to prevent drug release

in the stomach and provide controlled dissolution of enteric coat in the small intestine and maximum release in the colon. *in-vitro* drug release studies were performed in conditions simulating stomach to colon transit in the presence and absence of pectinase enzyme. The release of drug was found to be higher in the presence of pectinase enzyme. The effect of speed of stirred, concentration of polymer and reaction time were optimized on the basis of quality of beads and entrapment of beads.

25. **Hua Zhang *et al.*[98]** investigated a multiparticulate system of chitosan hydrogel beads for colonic delivery of macromolecules using fluorescein isothicyanate-labelled bovine serum albumin as a model protein. The results showed that the hydrogel beads were degraded by rat cecal and colonic enzymes, resulting in a marked increase in the release of protein which indicated the potential of the multiparticulate system to serve as a carrier to deliver macromolecules specifically to the colon. In their study, they utilized a commercially available almond emulsin beta-glucosidase preparation capable of degrading chitosan and that mimicked the degradation function of rat cecal and colonic enzymes in-vitro (**Zhang and Neau,** 2001**)** for checking the possibility of employing the enzyme preparation as a standardized in-vitro system to substitute for microbial enzymes to limit animal use for assessing the suitability of chitosan based formulations for colon targeting was tested.

26. **R. Mazumder *et al.*[102]** designed ethyl cellulose coated 5-flourouracil loaded pectin microspheres for colon targeting. The pectin microspheres were prepared by ionotropic-external gelation technique and ethyl cellulose coating was given by co-acervation phase separation method and characterization was performed by testing the entrapment efficiency, particle size, micromeritic properties, in-vitro release behaviour, SEM, FT-IR etc. In-vitro drug release study was performed in the presence of pectinase enzyme (Pectinex ultra SPL) which contains different pectinolytic and cellulolytic enzymes (endo-poly-galacturonase, endo-pectinylase and pectin esterase), and other activities such as ß-galactosidase, chitinase and transgalactosidase.

27. **Bala Venkata Pradeep.V *et al.*[11]** developed enteric coated pectin matrix tablets of Metronidazole for colon targeting. Matrix tablets were prepared by mixtures of pectin, a hydrophilic swellable polymer, in which the drug was dispersed. Eudragit S 100

used as a pH sensitive polymer for coating the matrix tablet. The drug release studies were performed by presence and absence of pectinolytic enzymes. The matrix tablets in presence of pectinolytic enzymes, undergoes a faster erosion process which, in agreement with the results of release studies, resulted in a more marked increase in drug release rate. Comparison of results obtained in the presence and absence of pectinolytic enzymes showed pectin was the most interesting candidate for colonic delivery and most susceptible to enzymatic degradation, thus assuring a greater site-specificity of drug release. From curve fitting the drug release showed good correlation coefficients (R values) for Korsmeyer-Peppas equation. The n value was found to be in between 0.85-1.26. The values are closer to 1 and hence it is concluded that the drug release was more dependent on the effect of polymer relaxation.

28. **KC Ofokansi *et al.*[63]** formulated and evaluated Gluteraldehyde-crosslinked Chitosan microparticles for the delivery of Ibuprofen, by using glueraldehyde saturated toluene (GST) as the cross-linking agent. The swelling behaviour of the particles and ibuprofen release were assessed in both simulated gastric fluid without pepsin (ph 1.2) and simulated intestinal fluid without pancreatin. Results have shown – Entrapment of Ibuprofen in gluteraldehyde-cross-linked Chitosan microparticles can be exploited to target and control the release of the drug and possibly reduce its gastro-erosive side effects.
29. **Surender Verma *et al.*[91]** formulated, evaluated and optimized osmotically controlled colon targeted drug delivery system, using microbially triggered and osmotically controlled approach,using Prednisolone as model drug for treatment of inflammatory bowel disease (IBD). Prednisolone-beta-cyclodextrin complex was prepared and phase solubility study was carried out. The formulations containing PEG was found to be promising drug delivery system better release kinetics.

30. **Gurunath.S.Padaonkar *et al.*[51]** have formulated and evaluated of oral novel colon targeted drug delivery system using natural polymers. This was used using pectin as carrier and Olsalazine sodium as model drug. The tablets were coated with inulin followed by shellac and were evaluated for average weight, hardness and coat thickness. The study revealed that polysaccharides as carriers and inulin and shellac as coating material can be used effectively for colon targeting of both water soluble and insoluble drugs .

31. **Morusu Keerthana *et al.*[86]** formulated and evaluated the Secnidazole colon targeting drug layered pellets. Secnidazole is used in treatment of Amoebiasis. The main objective is formulation of Secnidazole pellets by using power layering technique. Evaluation of prepared pellets can be performed by using parameters such as angle of repose, bulk density, carr's index and percentage drug release. By observing the percentage drug release studies of 5% and 10% concentration the 4 polymers one can conclude that the Eudragit RSPO 5% showed the better release compared to all other formulations.

32. **Sheth Zankhana *et al.*[69]** designed and developed 5-fluorouracil loaded biodegradable microspheres. The present study is aimed at the overall improvement in the efficacy, reduction in toxicity and enhancement of therapeutic index of 5-fluorouracil, by using solvent evaporation technique, by using polymethacrylate polymers like Eudragit L100,eudragit S100, eudragit P4135F and methylcellulose. Stabilty studies revealed that 4degree Celsius is the most suitable temperature for storage of 5-Fluorouracil. Overall, this study showed that the 5-fluorouracil can be formulated in a microparticulate drug delivery system by using various polymers and it showed significant prolonged drug release.

33. **N. Najmuddin *et al.*[65]** formulated and done in-vitro evaluation of floating microspheres of Ketoprofen prepared by emulsion solvent diffusion method using Eudragit S100 and Eudragit L100 polymer. The short half life of ketoprofen and multiple administration dose make ketoprofen a very good candidate for formulation of floating drug delivery system. Results show that as increase in drug: polymer ratio affects the particle size, percentage yield, *in-vitro* buoyancy and drug release of microspheres. The data obtained in this study thus suggest that a floating microspheres of ketoprofen are promising for sustained drug delivery which can reduce dosing frequency.

34. **Pornsak Sriamornsak *et al.*[83]** have done swelling and erosion of pectin matrix tablets and their impact on drug release behaviour. The matrix tablets were prepared by direct compression using different types of pectin. swelling and erosion studies were carried out in various media. The pectin matrix tablets formed a continuous gel layer while in contact with the aqueous media undergoing a combination of swelling

and erosion .The extent of matrix swelling, erosion and diffusion of drug determined the kinetics as well as mechanism of drug release from pectin-based matrix tablets . The findings were attributed to the effect of swelling and erosion on the drug release from hydrophilic matrix tablets, which is an interesting way of formulating oral sustained/controlled–release matrix tablets using a process that is easy and inexpensive and does not require special production equipment .

35. **Ranjit Singh.**[20] Formulated and evaluated the colon targeted delivery system for Nitrofurantoin using guar gum as carrier. When studies were continued in colon fluids, matrix tablets released almost 100% drug. The results of the study show that matrix tablets containing 20% guar gum are most likely to provide targeting of Nitrofurantoin in the colon.

36. **Erxi Che *et al.***[88] have developed phosphonate–terminated magnetic mesoporous silica nanoparticles for pH-controlled release of doxorubicin and improved tumor accumulation. Phosponate–terminated magnetic mesoporous nanoparticles was designed by incorporating MNPs in the centre of mesoporous silica nanoparticles and followed by grafting phosphonates group on the surface of MMSNs. The results can open the possibilities for combining magnetic targeting drug delivery system and pH-responsive release of doxorubicin to cancerous tissues.

37. **Heming Huang *et al.***[81] have done Mossbauer spectroscopy of protein-passivated iron oxide nanoparticles using uncoated and bovine serum albumin (BSA-)passivated magnetic iron oxide particles. Coating with BSA does not influence the particle morphology and does also not affect the magnetite to maghemite ratio.

38. **Meltum Cetin *et al.***[64] have prepared and characterized the anti cancer drug–loaded implantable PLGA (poly lactide –co-glycolide) microparticles. PLGA microparticles loaded with doxorubicin HCL (DOX) were prepared via o/w emulsion solvent evaporation. DOX-loaded PLGA microparticles displayed a significant cytotoxicity towards the RG2 cells as compared to the unloaded PLGA microparticles. This work can be considered for further studies on evaluation of the *in-vivo* efficacy and the brain's regional distribution of DOX-loaded PLGA microparticles using mature male Sprague-Dawley rats.

39. **Nitesh Shah *et al.*[21]** have designed, developed and optimized colon-targeted drug delivery system for Crohn's disease. The aim of the present investigation was to develop double coated systems comprising of pH independent (Eudragit RS100) and pH dependent coatings (Eudragit S100) of polymethacrylates for delivery of metronidazole exclusively to the colon. Metronidazole colon targeted tablets prepared in the present work by double coating method. Optimization of coating levels of both the coats using central composite design reveals that the polymer concentration and coating level plays a significant role in the drug release property of which coating level of Eudragit RS was more significant after the tablet reaches colon. Thus proposed system may be successfully used for colon targeting of Metronidazole.

3.1 AIM OF THE WORK:

Mesalamine (5- amino salicylic acid) is an anti-inflammatory drug used for the first line treatment of Inflammatory Bowel Diseases (IBD); namely Ulcerative Colitis (UC) and Chron's Disease (CD).

The aim of the present study is:

- To formulate Magnetically Responsive Mesalamine Microspheres by solvent evaporation method by using biodegradable polymers Chitosan and Pectin with an intention to produce a depot near the target organ and drug delivery utilizing the principle of magnetic targeting.
- To carry out the various pharmaceutical and magnetic characterizations and thereby to optimize the formulation and to study the effect of polymer type on *in- vitro* drug release
- To carry out the *in-vitro* release studies using microflora activated system.
- To carry out the *in-vitro* anti-inflammatory activity by inhibition of protein denaturation method.

The main objective of the study is to localize the drug only at the target site and thereby minimizing the dose and drug induced toxicity.

3.2 PLAN OF THE WORK:

The present study was designed and planned as follows:

1. **Literature Survey or Review of Literature**
2. **Preformulation studies**
   - ✓ FTIR study- Identification and Compatibility of drug and excipients.
3. **Standard Curve for Mesalamine in Phosphate Buffer Saline pH 7.4.**
4. **Formulation and Development**
   - ✓ Chemical synthesis of the magnetic carrier – Magnetite (Fe_3O_4).
   - ✓ Formulation of Magnetically Responsive Microspheres of Mesalamine using biodegradable polymers (Chitosan and Pectin) in different drug-polymer ratios.

5. Evaluation studies of Magnetic Microspheres

5.1 Physicochemical Characterization

- Surface morphology
- Mean Particle Size
- Drug Excipient Interaction – FT-IR

5.2 Pharmaceutical Characterization

- Percentage Yield
- Drug content (%)
- Drug Loading (%)
- Swelling Kinetics
- Evaluation of Flow Property
- *in-vitro* drug release study of Microspheres
- Study of effect of polymer type on *in- vitro* drug release
- Release kinetics of the optimized formulation
- Stability Studies of the Optimized formulation- at ambient conditions and accelerated conditions as per the ICH guidelines

5.3 Preclinical *in-vitro* Screening Studies

- *in-vitro* release study of optimized formulation using microflora activated system
- *in-vitro* anti-inflammatory activity of optimized formulation

5.4 Magnetic Characterization

- Magnetite Content Determination
- Magnetic Separation Behaviour
- Magnetism Assesment using VSM

4. RATIONALE OF THE STUDY

Irritable Bowel Diseases (IBD) are the chronic idiopathic multi-factorial inflammatory diseases of gastrointestinal tract, which mainly include two forms; Ulcerative Colitis (UC) and Chron's Disease (CD).[15]

IBD which has been ranked 5th among the most prevalent gastro intestinal diseases and if left untreated posses a high risk to develop as colon cancer.[15] IBD can affect a person of any age and can continue throughout their life as it does not have any permanent cure.

Treatment objectives are:

1. Reduction in drug related side effects by employing site-specific local targeting.
2. Improve the quality of life.

4.1 RATIONALE FOR SELECTION OF DRUG

Small intestine and large intestine or colon are the main regions involved in IBD, marked by chronic inflammation in specific mucosal or transmural locations as a result of an immune reaction of the body against its own intestinal tissue[15].

The available conventional dosage forms are not effective in the treatment of this colonic disease as dosing frequency is quite high which leads to many adverse effects. Drug delivery systems for treating the colonic disorders such as IBD are failing as the drugs do not reach the site of action in appropriate concentration. Thus, there is a need to develop effective and safe therapy for the treatment of these colonic disorders, using site- specific targeted drug delivery approach.

The drug of choice, Mesalamine is an intestinal anti-inflammatory drug, amino salicylic acid derivative. It acts by reducing inflammation of colon topically by inhibiting the production of IL-1 and TNF-α, inhition of the lipoxygenase pathway, scavenging of free radicals and oxidants, and inhibition of NF-κB.[15] Mesalamine undergoes extensive first pass metabolism. Adult dose is about 800 mg two- three times a day for total of 2.4 g/day. Thus, the development of site-specific targeted drug release to colon would be clearly advantageous.[73]

4.2 RATIONALE FOR SELECTION OF POLYMER [10,88,62,63,67]

Chitosan and Pectin are biodegradable polymers which undergo enzymatic degradation in colon resulting in drug release in colonic pH. When combined with the principle of magnetic carrier technology, these polymeric carriers act as a smart biodevice for site specific colon targeted drug delivery.[10] Both these polymers used in different ratios are cheap, non toxic, biocompatible and have controllable biologic activity.

4.3 RATIONALE FOR SELECTION OF MAGNETIC CARRIER[10]

In this study, ferric oxide (Fe_2O_3) is used as the preferred magnetic carrier because of its non-toxicity, superparamagnetism (for magnetite particles below 30-40 nm),[49] high level of accumulation in tissues, interruption of magnetization when the magnetic field is removed, and biocompatibility due to high affinity for water which allows to interact with biological species.[10]

4.4 RATIONALE FOR SELECTION OF DOSAGE FORM[43,16]

Upon oral administration, 5-ASA exhibits rapid and nearly complete absorption from the upper intestine, resulting not only in systemic side effects but also in lowering the dose reaching the colon with the subsequent decreased probability of therapeutic success.[92]

Drug delivery systems based on microparticles sensitive to a remotely applied magnetic field appear on the top of the (bio) technological innovations, because the magnetic field, if used in a therapeutic level, does not affect biological tissues.[10]

The present study is to formulate Magnetically Responsive Mesalamine Microspheres which offers a localized drug delivery only at the target site by the combined effect of **physical approach** (utilizing the principle of magnetic targeting with an intention to produce a depot near the target organ) and **biochemical approach (**using biodegradable polymers chitosan and pectin for drug release in a controlled manner).[17] By producing a depot near the target organ, unwanted distribution of drug to non target organ can be avoided. This approach will localize the drug only at target site and minimize the dose and drug induced toxicity.

5.1 DISEASE PROFILE

5.1.1 Irritable Bowel Disease (IBD) [15,23,43,50,79]

Irritable Bowel Diseases (IBD) are the chronic idiopathic multi-factorial inflammatory diseases of gastrointestinal tract, which mainly include two forms; Ulcerative Colitis (UC) and Chron's Disease (CD). Small intestine and large intestine or colon are the main regions involved in IBD, marked by chronic inflammation in specific mucosal or transmural locations as a result of an immune reaction of the body against its own intestinal tissue.

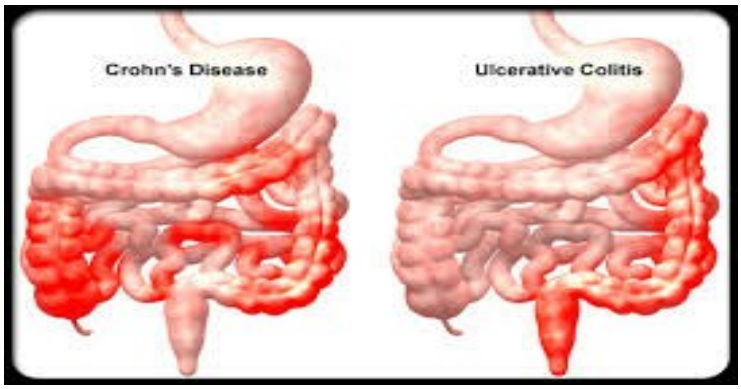


Figure 14: Chron's Disease & Ulcerative Colitis

Depending on the location of disease, UC can be further classified as[43]

- Ulcerative Proctitis (disease only in rectum)
- Limited or Distal Colitis (disease in the left side of the colon)
- Pancolitis (disease in the entire colon).
- Proctosigmoiditis

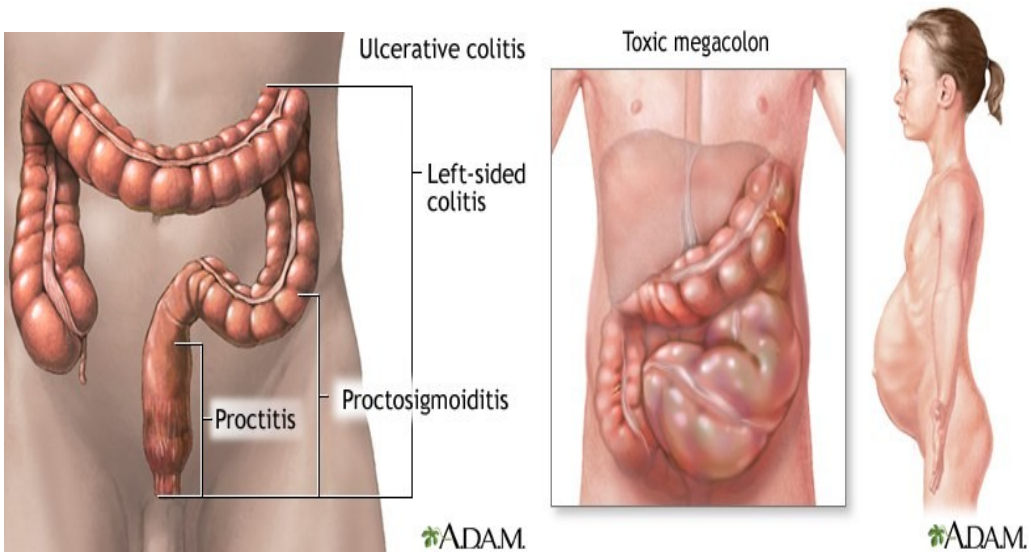


Figure 15: Types of Ulcerative Colitis

Accounting for far fewer cases are the other forms of IBD:

- Collagenous Colitis
- Lymphocytic Colitis
- Ischaemic Colitis
- Diversion Colitis
- Behcet's Colitis
- Indeterminate Colitis

5.1.2 Incidence and Prevalence[15]

Irritable Bowel Disease is becoming a global disease because of high incidence and prevalence of it with respect to time. IBD occupied 5th rank among the most prevalent gastrointestinal diseases and if left untreated posses a high risk to develop as colon cancer.[25] It has been estimated that upto 2,40,000 people in U.K, 2.2 million in Europe and 1.4 million in United States are suffering from IBD, among which, 100,000 are children. There are very lower incidences in Asia, Africa and South America. Peak incidences occur in young people between 15–40 years of age. In general IBD can affect a person of any age and can continue throughout their life as it does not have any permanent cure.

5.1.3 Etiology of IBD[15]

The etiology of IBD is unknown. There are three interrelated factors thought to be affect symptoms to varying degrees:

- ***Gut factors***
- ***Environmental factors***
- ***Genetic factors***

5.1.3.1 Gut factors

- **Dysregulation of immune system**: It leads to excessive immune response to normal microflora and epithelial cell abnormalities. Changes in the composition of gut microflora facilitate abnormal inflammatory responses.
- **Microbial infections**: Mycobacterium paratuberculosis, measles virus and bacterial flora have been suggested as possible causes of CD.

- **Impaired epithelial barrier function**: It leads to abnormal intestinal permeability which further results in
- **Impaired gene expression**: by enhanced apical expression of transferring receptor proteins in enterocytes in inflammatory mucosa of IBD patients.

5.1.3.2 Environment factors

- **Smoking**: The risk of developing CD is high in smokers.
- **Oral contraceptives:** There may be a slight association between oral contraceptive use and the development of CD.
- **Diet:** Diet high in fat and sugars, Stress etc may also contribute to IBD.

5.1.3.3 Genetic factors

- **Family history:** One in five people with IBD have a first-degree relative (parent, child or sibling) with the disease.
- **Race and ethnicity:** UC is more common among whites than in non-whites..it also occurs mostly in Caucasians. There is also evidence for ethnic aggregation (higher in Jewish decent).
- **Genes linked to IBD:** recently, the first gene associated with CD, the NOD2 was identified.

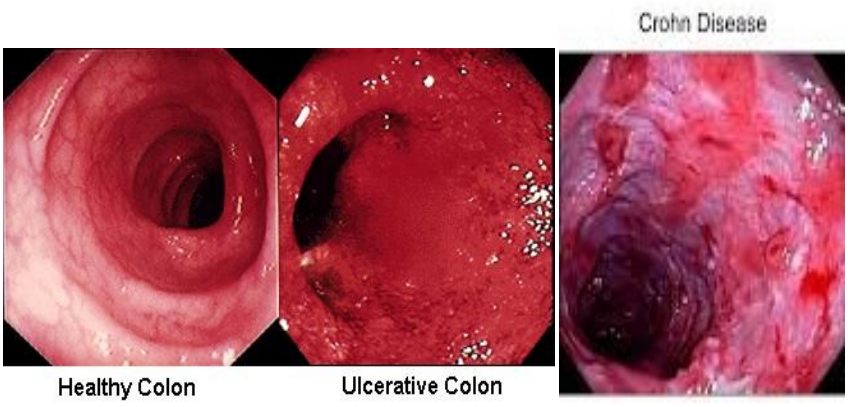


Figure 16: Colon affected by Chron's Disease & Ulcerative Colitis

Ulcerative Colitis versus Crohn's Disease

The basic distinction between UC and CD are location and severity. However, some patients with early stage IBD have features and symptoms of both disorders (called indeterminate colitis). In general, UC is a local inflammatory bowel disorder in which continuous mucosal inflammation extends from the rectum towards the cecum, and is generally associated with excess IL-13 production whereas, CD is a relapsing inflammatory disorder which may involve any part of the GIT but more pronounced in ileocecal region which is generally associated with excess production of IL-2/IL-23 and IFN-γ/IL-17. These Inflammatory Bowel Diseases have been linked with an increased risk of colorectal cancer.

Table: 6 Comparison of Crohn's vs. Ulcerative Colitis[15]

S.No	Features	Ulcerative Colitis	Crohn's Disease
1	Site of action	Extending from rectum proximally to entire colon only	Any part of GIT (mainly ileum is involved)
2	Symptoms	Diarrhea, weight loss, malnutrition and other extraintestinal manifestations, smoking improves condition	Diarrhea, abdominal pain, weight loss, growth failures in kids, smoking worsens the condition
3	Pathology and complications	Mucosal inflammation, if get severe can cause colon cancer. Fistulas, abscesses and strictures absent	Transmural inflammation, non caseating granuloma, fistulas, abscesses, perianal involvement and strictures are common, toxic megacolon absent
4	Cytokine response	Associated with T_h17	Associated with T_h2
5	Distribution	Continuous distribution	Discontinuous
6	Drugs used	5-ASA, Sulfasalazine, Balsalazide, Infliximab, Azathioprine and mercaptopurine	Hydrocortisone, Budesonide, Prednisolone, Sulfasalazine, Olsalazine, Mesalazine and Balsalazide, Infliximab

5.1.4 SYMPTOMS AND COMPLICATIONS[43]

Intestinal

- Abdominal cramps and pain
- Bloody diarrhoea
- Severe urgency to have a bowel movement
- Fever
- Loss of appetite, Weight loss, Anaemia (due to blood loss)

Intestinal complications of IBD includes:

- Profuse bleeding from the ulcers.
- Perforation (rupture) of the bowel.
- Strictures and obstruction: more common in persons with CD.
- Fistulae and perianal disease: more common in persons with CD.
- Toxic megacolon: this life-threatening complication of UC requires surgery
- Colorectal cancer: there is high risk of colon cancer in patients with UC. Chronic inflammation generated by the production of reactive oxygen species leads to generate dysplasia, which further develops into CAC, i.e., colitis associated colorectal cancer, which is a severe form of ulcerative colitis.

Extraintestinal

Persons with IBD may have

- Arthritis
- Osteopenia (low bone density), Osteoporosis (bone loss)
- Impaired skeletal and groth development in children due to malabsorption and malnutrition.
- Liver and kidney disorders

5.1.5 PATHOPHYSIOLOGY [15,79]

Multiple etiologies have been proposed for IBD, but the precise cause is unknown. When a luminal antigen crosses the epithelial layer, T- lymphocytes (helper cells and cytotoxic cells) are activated. These t-cells are normally found in gut wall but in IBD, the normal regulation of their activity is disturbed. Helper T-cells, type-1 (Th-1), are associated principally with CD, whereas Th-2 cells are associated principally with UC.

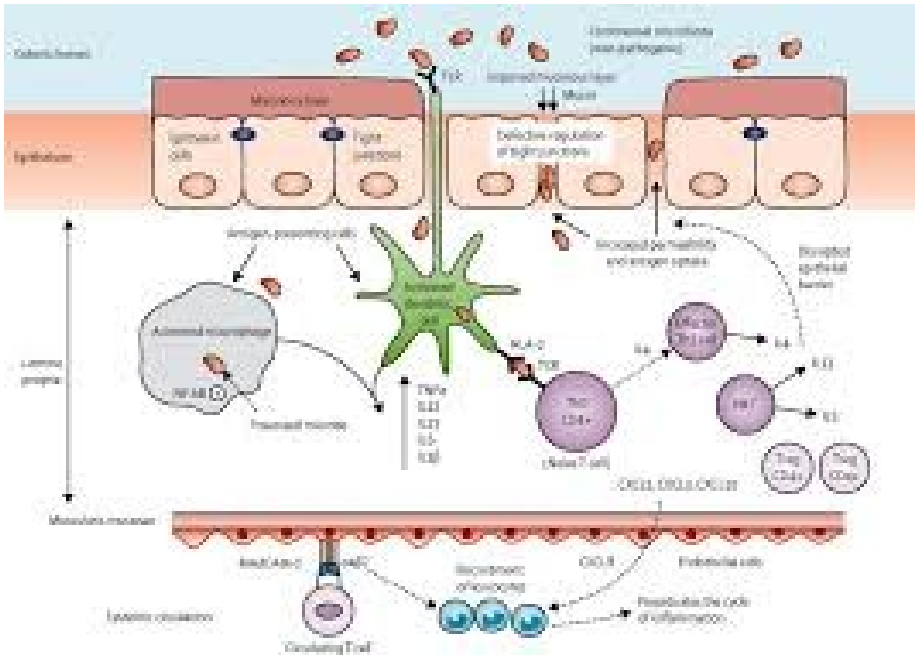


Figure 17: Pathophysiology of IBD

In UC, with the influx of neutrophils in lamina propia, there is localized collection of pus cells surrounded by inflamed tissues and causes depletion of mucin. Activation of mucosal inflammatory cells also leads to the production of large number of inflammatory mediators such as cytokines, leukotrienes, prostaglandins, platelet activating factor, oxygen radicals, thromboxanes, proteases etc. which are atleast partly responsible for tissue damage.

In CD, the accompanying inflammation is described as irregular/ patchy, segmented, and transmural. Most commonly, terminal ileum exhibits early lesions on or near Peyer's patches.[15]

5.1.6 MAJOR CHALLENGES IN THE TREATMENT OF IBD[15]

Cure of UC depends on severity of disease its subtypes and its pre-existing illness. Long term medication produces lot of side effects such as peptic ulcers, diarrhea, nephrotoxicity and hepatotoxicity, glaucoma, vomiting, Cushing's syndrome etc., which negatively affects the quality of life of UC suffered patients.

Major challenge in the treatment of UC is the reduction in drug related side effects by employing site specific local targeting.

5.1.7 TREATMENT OF IBD [15,23]

Most treatments for IBD are either medical or surgical. Surgery is typically used only if all the medical options have failed. The goal of medical treatment in IBD is to suppress the abnormal inflammatory response. This allows the intestinal tissue to heal, relieving the symptoms of diarrhea and abdominal pain. The recommended first-line therapy for the treatment of active symptoms, induction of remission and maintenance of remission in patients with mild to moderate UC is the anti-inflammatory agent 5-aminosalicylic acid (5-ASA).

Pharmacological therapy includes:

- Aminosalicylates
- Corticosteroids
- Immunosupressants
- Anti-tumor necrosis factor (TNF) agents and
- Antibiotics.

Table: 7 Drugs used for the treatment of Ulcerative colitis[15]

S. No	Classes of drugs		Trade name	Marketed formulations	Adverse effects	Dose
1	**Aminosalicylates**	Sulfasalazine	Azulfidine	Delayed release tablet	Agranulocytosis, panceatitis, intestinal nephritis	4-6g/day divided qid
		Mesalamine	Asacol	Eudragit S100 coated tablet (dissolves at pH 7)	Hepatits, male infertility, arthralgia, pneumonitis, loss of apetite, stomach upset, blurred vision	2-4g/day divided qid
		Balsalazide	Colazal	Tablet	Head ache, abdominal pain, stomach pain, stomach upset, diarrhea, vomiting, joint pain.	1.5-3g/day divided qid

2	Corticosteroids	Budesonide	Entocort	Eudragit L coated beads	Dry or irritated mouth or throat,cough, painful speech, neck pain, stomach pain	
		Prednisolone	Deltasone, Orasone	Tablets of 2.5,5,10,20 and 50mg and oral solutions/ syrups	Hyperglycaemia, hypertension, electrolyte disturbances	5-50mg/ day
		Dexamethasone	Dexasone, diodex, decadron	Tablets, elixirs and solutions	Cataract, osteoporosis,myopathy	Tablets 0.25-6mg , Elixir 0.5mg/ml, Solution 0.5,1mg/5ml
3	Immunosupressants	Azathioprine, Methotrexate	Imuran, Rheumatrex, Traxell	Tablet, Solution given i.v	Hyperglycaemia, hypertension, electrolyte disturbances, cataract	50mg 25mg/ml
4	Antimicrobials	Metronidazole	Flagyl	Extended release tablets 750mg, capsule 375mg, cream 0.75 %and 1% injections	Utricaria, glossitis,long term use may develop paresthesia	750mg orally tid for 5- 10 days
		Ciprofloxacin	Cipro, Proquin XR	Tablets 250,500 and 750mg, Extended release (XR) 500 & 1000mg, injections 200mg/100ml	Diarrhea, vomiting and rash. Other side effects (e.g. head ache, abdominal pain, cardiovascular, gastrointestinal, etc.)in less than 1% of the patients	500mg twice daily
5	Anti-TNF agents	Infliximab	Remicade	Powder for intravenous injection	Acute infusion reactions	100mg
		Adalimubab	Humira	Prefilled glass syringe	Serum sickness, increase in sepsis, pneumonia, tuberculosis etc.	20mg/ 0.4 ml

5.1.8 DIAGNOSTIC PROCEDURES[43]

To help confirm diagnosis of CD or UC, one or more of the following tests and diagnostic procedures may be performed.

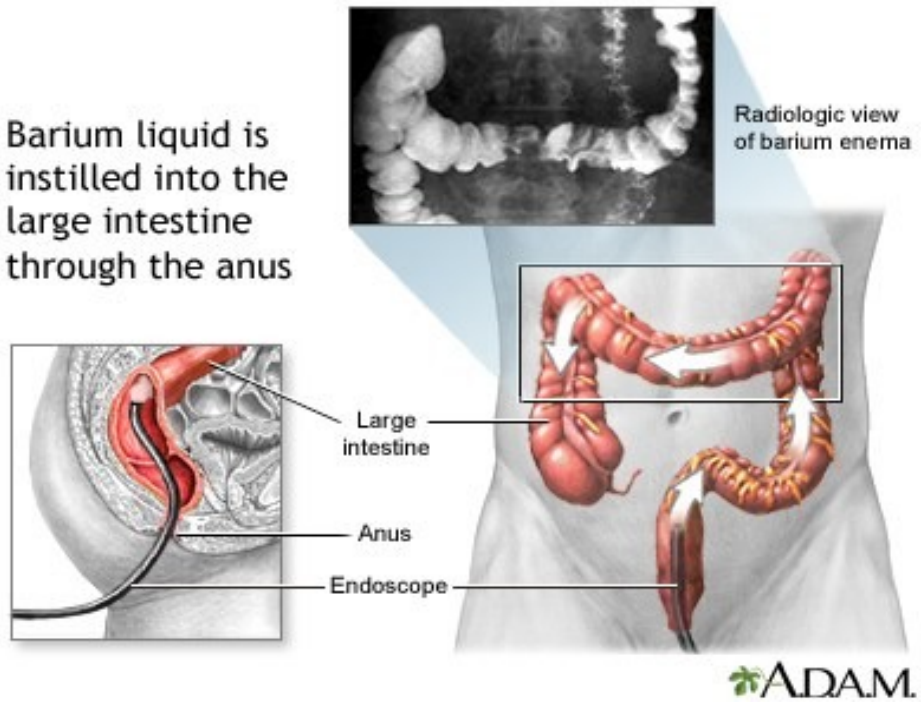


Figure 18: Diagnosis of IBD

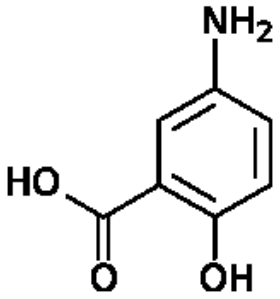
- ***Blood tests:*** the presence of inflammation in the body can be identified by the abnormal levels of red and white blood cells, platelets and C-reactive protein (CRP).
- ***Stool tests:*** it checks for signs of inflammation in GIT as well as infections.
- ***Endoscopic procedures***: includes Flexible sigmoidoscopy and colonoscopy.
- ***External imaging procedures:*** e.g. computerized tomography (CT) scans and magnetic resonance imaging (MRI).

6.1 MESALAZINE[32,79,109]

Mesalazine is an intestinal anti-inflammatory drug, amino salicylic acid derivative, used in the treatment of ulcerative colitis. It acts by reducing inflammation of colon topically by preventing production of substances involved in inflammatory process such as arachidonic acid.

6.1.1 PHYSIOCHEMICAL PROFILE:

Chemical Structure



Molecular Formula : $C_7H_7NO_3$

Molecular Weight : 153.1

Chemical Name : 5-Amino-2-hydroxybenzoic acid

CAS : 89-57-6

Category : Gastrointestinal Agents, Anti ulcer

Description : white or light grey or light pink powder or crystals.

Solubility : Very slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides and in dilute hydrochloric acid.

TAXONOMY

Kingdom : Organic Compounds

Super Class : Benzenoids

Class : Benzene and substituted derivatives

Sub Class : Benzoic acids and derivatives

Direct Parent : Aminobenzoic acids

Melting Point : 275-280^{o}C .

Packing and Storage : in an airtight container, protected from light.

6.1.2 PHARMACOKINETIC PROFILE [23,59,109]

ABSORPTION

5-ASA undergoes a complete systemic absorption within the proximal intestine when administered in an unformulated fashion, leaving little or no 5-ASA to treat the colon. Mesalamine is quickly metabolized by N-acetyl-transferase 1 to N-acetyl-5-ASA within intestinal epithelial cells and the liver**.** Oral bioavailability: 20-30% is absorbed; 10-35% absorbed in the colon.

DISTRIBUTION

About 80% of N-Acetyl-5-amino salicylic acid is bound to plasma proteins, whereas 40% of mesalamine is protein bound and the volume of distribution data is not available.

METABOLISM

Rapidly and extensively metabolized, mainly to N-acetyl-5-ASA (Ac-5-ASA) in the intestinal mucosal wall and the liver. Ac-5-ASA is further acetylated (deactivated) in at least 2 sites, the colonic epithelium and the liver.

EXCRETION

It is extensively metabolized in the liver and it is excreted in the urine and faeces as either unmetabolized 5-ASA or N-acetyl-5-ASA. The half-life of mesalamine is dose-dependent and reaches up to 1.4 h while the half-life of N-acetyl-5ASA reaches up to 6 h.

6.1.3 PHARMACOLOGICAL PROFILE

MECHANISM OF ACTION

Although mesalamine is a salicylate, its therapeutic effect does not appear to be related to cyclooxygenase inhition; possible mechanism of action by inhibition of the production of IL-1 and TNF-ά, inhition of the lipoxygenase pathway, scavenging of free radicals and oxidants, and inhibition of NF-κB. Specific mechanism of action of this drug have not been identified.[15]

INDICATION AND USAGE:

Used in the treatment of Inflammatory Bowel Diseases such as

- Ulcerative colitis (mild to moderate)
- Crohn's disease

DOSAGE (FOR ADULTS):

Controlled release tablets	:	**PO** 800 mg tid for total of 2.4 g/day for 6 week.
Suppositories	:	**PR** 500 mg suppository bid for up to 6 week. Retain suppository in rectum for 1–3 hr or more to achieve maximum benefit.
Suspension Enema	:	**PR** 4 g in 60 ml as rectal instillation qid for up to 6 week, preferably at bedtime, retained for 8 hr.

SIDE EFFECTS:

Cardio vascular	:	Chest pain.
CNS	:	Headache; asthenia; dizziness; fever; sweating; malaise.
DERM	:	Acne; itching; rash.
EENT	:	Rhinitis; sore throat; pharyngitis.
GI	:	Abdominal pain; cramps; discomfort; colitis exacerbation; constipation; diarrhoea; dyspepsia; vomiting; flatulence; nausea
RESP	:	Cough.

PRECAUTION:

Pregnancy	: Category B.
Lactation	: Excreted in breast milk.
Children	: Safety and efficacy not established.

Intolerance and colitis exacerbation:

Some patients develop acute intolerance syndrome or exacerbation of colitis characterized by cramping, acute abdominal pain, bloody diarrhoea etc. Symptoms generally abate when mesalamine is discontinued.

CONTRAINDICATION:

Hypersensitivity to salicylates

OVERDOSE:

In case of overdose atrioventricular block, cardiac arrest paralysis of respiratory center may occur. The treatment is symptomatic.

DRUG INTERACTIONS:

Reduced absorption of digoxin has been reported when administered concomitantly with Mesalamine.

BRAND NAMES:

Asacol	by Win-Medicare Limited
Pentasa -500	by Ferring Pharmaceuticals
5A	by Wallace Pharmaceuticals Ltd.
Mesacol (80mg)	
Mesacol Suppos.	by Sun Pharmaceuticals
Mesacol Enema	

7.1 CHITOSAN[48,66]

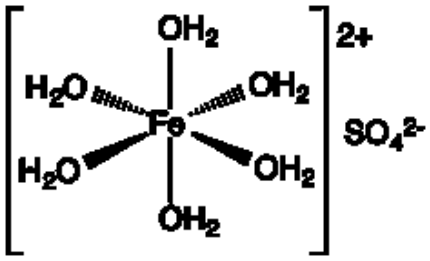
Nonproprietary Names	**BP**: Chitosan Hydrochloride **PhEur**: ChitosaniHydrochloridum
Synonyms	2-Amino-2-deoxy-(1,4)-ß-D-glucopyranan; deacetylated chitin; deacetyl chitin; ß-1,4-poly-D-glucosamine; poly-D-glucosamine; poly-(1,4-ß-D-glucopyranosamine).
Chemical Name	Poly-ß-(1,4)-2-Amino-2-deoxy-D-glucose.
Empirical Formula & Molecular Weight	Chitosan is chitin deacetylated to form soluble. Mol. Wt: 10,000 – 10,00,000**.**
Description	Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation during precipitation (cottonlike chitosan).
Moisture content	Adsorbs moisture from the air, the amount depends on the initial moisture content, temperature and relative humidity**.**
Solubility	Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents and neutral or alkali solutions at pH above 6.5. dissolves readily in dilute and conc. solutions of most organic acids and to some extent I mineral inorganic acids.
Functional Category	Coating agent; disintegrant; film-forming agent; mucoadhesive; tablet binder; viscosity-increasing agent.
Applications in Pharmaceutical Formulations	Chitosan is used in cosmetics and is under investigation for use in drug delivery applications like controlled drug delivery, mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery.

7.2 PECTIN[48,66]

Nonproprietary Names	USP: Pectin
Synonyms	Citrus Pectin; E440; methopectin; methyl pectinate; mexpectin; pectin; pectinic acid
Chemical Name	Pectin.
Description	Pectin is a high molecular weight, carbohydrate- like plant constituent consisting primarily of chains of galacturonic acid units linked as 1,4-a-glucosides.
Molecular Weight	Mol. Wt: 30,000 – 1,00,000.
Description	Pectin occurs as a coarse or fine, yellowish- white, odorless powder that has a mucilaginous taste.
Solubility	Soluble in water; insoluble in ethanol (95%) and other organic solvents
Functional Category	Adsorbent; emulsifying agent; gelling agent;thickening agent; stabilizing agent.
Applications in Pharmaceutical Formulations	Pectin is used as an adsorbent, bulk forming agent and emulsion stabilizer. Pectin gel beads have been shown to be effective for controlling the release of a drug within the GIT. It is used in colon- biodegradable pectin matrix with a pH-sensitive polymeric coating, which retards the onset of drug release, overcoming the problems of pectin solubilityin the upper GIT. Pectin-based matrices with varying degrees of esterification as oral controlled- release tablets.

7.3 FERROUS SULPHATE[32,72]

IUPAC Name	Iron(II) sulphate
Synonyms	Green vitriol, Iron vitriol, Copperas, Melanterite, Szomolnokite
Chemical Formula& CAS No	$FeSO_4.7H_2O$ [7782-63-0]



Molecular Weight & Density	278 [1.895g/cm^3]
Melting Point	333 – 337 K
Description	Blue-green crystals or light green, crystalline powder; odorless, efflorescent in air. On exposure to moist air, the crystals rapidly oxidize and become brown.
Solubility	Soluble in water (29.51 g/100ml at 25^0 C); insoluble in alcohol.
Vapor Pressure	1.95 kPa
Magnetic susceptibility(χ)	$1.12x10^{-2}$ cm^3/mol
Refractive Index (η_D)	1.471
Functional Category	Haematinic
Dose	Prophylactic, 300mg; therapeutic 600 to 900mg daily, in divided doses. (300mg of ferrous sulphate is approximately equivalent to 60 mg of ferrous iron).

7.4 SODIUM HYDROXIDE[48]

Nonproprietary Names	**BP**: Sodium Hydroxide **JP**: Sodium Hydroxide **USP-NF**: Sodium Hydroxide **PhEur**: Sodium Hydroxide
Synonyms	Caustic soda, E524, lye, soda lye, sodium hydrate
Chemical Name & CAS	Sodium hydroxide [1310-73-2]
Empirical Formula & Molecular Weight	NaOH Mol. Wt: 40.00
Description	Sodium hydroxide occurs as white or nearly white fused mass, available in small pellets, flakes or sticks, hard and brittle and shows a crystalline fracture, very deliquescent and on exposure to air it rapidly absorbs carbondioxide and water.
Typical Properties	*Acidity/ alkalinity* pH approx. 12 (0.05% w/w aqueous solution) pH approx. 13 (0.5% w/w aqueous solution) pH approx. 14 (5% w/w aqueous solution)
Functional Category	Alkalizing agent, buffering agent
Applications in Pharmaceutical Formulations	It is widely used to adjust pH of solutions**.** It can also be used to react with weak acids to form salts.

7.5 MAGNESIUM STEARATE[48]

Nonproprietary Names	**BP**: Magnesium Stearate **JP**: Magnesium Stearate **USP-NF**: Magnesium Stearate **PhEur**: Magnesii Stearas
Synonyms	Magnesium octadecanoate; octadecanoic acid; magnesium salt; stearic acid.
Chemical Name & CAS	octadecanoic acid magnesium salt.
Empirical Formula &	**$C_{36}H_{70}MgO_4$**
Molecular Weight	591.34
	The **USPNF** describes magnesium stearate as a compound of magnesium with a mixture of solid organic acidsthat consists chiefly of variable proportion of magnesium stearate and magnesium palmitate. The PhEur 2005 describes it as amixture of magnesium salts of different fatty acids chiefly stearic and palmitic acids and in minor proportions of other fatty acids.
Description	It is a very fine, light white, precipitated or milled, impalpable powder of low bulk density, having a faint stearic acid odour and a characteristic taste. It is greasy to touch and readily adheres to skin.
Solubility	It is practically insoluble in ethanol, ether and water; slightly soluble in warm benzene.
Functional Category	Tablet and capsule lubricant.
Applications in Pharmaceutical Formulations	It is widely used in cosmetics, food and pharmaceuticals It is primarily used as a lubricant in capsule and tablet manufacture.

7.6 LIQUID PARAFFIN[48]

Nonproprietary Names	**BP**: Light Liquid Paraffin **JP**: Light Liquid Paraffin **USP-NF**: Light Mineral Oil **PhEur**: Paraffin, Light Liquid
Synonyms	Light white mineral oil
Chemical Name	Light mineral oil
Empirical Formula	It is a mixture of refined liquid saturated hydrocarbons obtained from petroleum. It is less viscous and has a lower specific gravity than mineral oil
Molecular Weight	
Description	Light mineral oil is a transparent, colorless liquid, without fluorescence in daylight. It is practically tasteless and odorless when cold, and has a faint odor when heated.
Typical Properties	Soluble in chloroform, ether and hydrocarbons, sparingly soluble in ethanol; practically insoluble in water.
Functional Category	Emollient; oleaginous vehicle; solvent; tablet and capsule lubricant; therapeutic agent.
Applications in Pharmaceutical Formulations	It is widely used to adjust pH of solutions**.** It can also be used to react with weak acids to form salts.

8. MATERIALS AND METHODS

8.1 MATERIALS USED IN FORMULATION

The list of drugs and excipients, their manufacturer and use in the present study are shown in Table 8

Table 8- List of drug(s) and excipients

Name of the material	Name of the company	Use in the formulation
Mesalamine	IPCA Laboratories, MP	Active Ingredient
Chitosan	Sigma Aldrich	Biodegradable polymer
Pectin	HiMedia Laboratories Pvt. Ltd, Mumbai	Biodegradable polymer
Magnesium Stearate	MMC Health care, Chennai	Droplet stabilizer
Talc	MMC Health care, Chennai	Stabilizing agent
Liquid Paraffin	Microfine Chemicals, Chennai.	Dispersion phase
Sodium Hydroxide	IRP, Chennai	Reagent
Ferrous Sulphate	Microfine Chemicals, Chennai	Reagent
Tween 80	Supra Chemicals, Chennai	Emulsifier
Span 80	Supra Chemicals, Chennai	Emulsifier

Bovine albumin	Supra Chemicals, Chennai	Reagent
ß-glucosidase	Synkromax, Chennai	Enzyme preparation
Pectinase	Synkromax, Chennai	Enzyme preparation
Potassium dihydrogen ortho Phosphate	Supra Chemicals, Chennai	Reagent
Disodium hydrogen phosphate	Supra Chemicals, Chennai	Reagent
Sodium chloride	Supra Chemicals, Chennai	Reagent
Hydrochloric acid	Supra Chemicals, Chennai	Reagent
Glacial acetic acid	Supra Chemicals, Chennai	Solvent
Dist. Water	Supra Chemicals, Chennai	Solvent
Gluteraldehyde	Microfine Chemicals, Chennai	Cross linking agent
Toluene	Supra Chemicals, Chennai	Solvent
n-Hexane	Supra Chemicals, Chennai	Solvent
Span 80	Microfine Chemicals, Chennai	Emulsifier

8.2 EQUIPMENTS

Table 9- Equipments used in the formulations and evaluation of Microspheres

Name of the equipment	Name of the company
High Speed Homogenizer	Remi, India
Centrifuging Machine	MC Dalal, Chennai.
Electronic Balance	MC Dalal, Chennai.
UV Spectrophotometer	Shimadzu, Japan
Optical Microscope	Focus Trademark, India
Binocular Microscope	Olympus, India.
SEM Analyser	Hitachi, Japan
FT- IR Spectrophotometer	Nicolet, India
Dissolution Apparatus	Campbell, India
Vibrating Sample Magnetometer	Lakeshore, USA
BOD Incubator	CDS Lab, Ambala Cantt
Stability Chamber	REMI CHM-6-plus

8.3 PREFORMULATION STUDIES[11,24]

The preformulation studies are the first step in the rational development of any formulation. It can be defined as "investigation of physical and chemical properties of drug substance alone and combined with the excipients. "The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass produced.

The goals of the study are:

- To establish physical characteristics.
- To establish its compatibility with the excipients.
- To determine kinetic rate profile.

8.4 DRUG-POLYMER INCOMPATIBILITY STUDIES

8.4.1 Fourier Transform Infra-Red Spectroscopy[24,34]

The compatibility between pure drug and polymer was detected by FT- IR spectra obtained. 1-2mg of Mesalamine alone, mixture of drug and excipients were weighed and mixed properly with Potassium bromide uniformly. The spectra's were recorded over the wave number 4000- 500cm^{-1}.

Table 10- Composition of drug and excipients for FTIR spectra

S.No.	Ingredients
1	Drug alone
2	Drug & Chitosan
3	Drug & Pectin
4	Drug & Magnetite
5	Drug & Magnesium stearate

8.5 CALIBRATION CURVE FOR MESALAMINE[53,86]

8.5.1 Preparation of Phosphate Buffer Saline (PBS) pH 7.4[32]

Dissolve 2.38g of disodium hydrogen phosphate, 0.19g of potassium dihydrogen phosphate and 8.0g of sodium chloride in sufficient water to produce 1000ml. Adjust the pH, if necessary.

8.5.2 Preparation of 0.1N Hydrochloric acid (pH 1.2)[32]

8.5ml of conc. HCl was dissolved in 1000ml of distilled water. Adjust pH, if necessary.

8.5.3 Standard Curve in Phosphate Buffer Saline pH 7.4[32,53,86]

100 mg of Mesalamine was transferred into a volumetric flask and dissolved in 15ml of 0.1N hydrochloric acid and the volume was made up to 100ml with PBS pH 7.4. The resulting solution was labeled as stock solution 1. From this stock solution, 10ml was taken and diluted to 100ml with Phosphate buffer saline pH 7.4 was labeled as stock 2. From this stock solution, 4ml, 8ml, 12ml, 16ml, 20ml, 24ml and 28ml were pipetted out into separate standard flasks and made up to 100ml with Phosphate buffer saline pH 7.4. The absorbance of solution is measured at 230nm using UV- Visible Spectrophotometer. The calibration curve was then plotted taking concentration on X- axis and absorbance on Y- axis.

8.6 PREPARATION OF MESALAMINE MAGNETIC MICROSPHERES

8.6.1 Preparation of Magnetite[28]

The magnetite (Fe_3O_4) was prepared by reacting 10%w/v ferrous sulphate (containing 5% tween 80) with 20%w/v sodium hydroxide solution, followed by washing of the precipitate with dilute ammonia inorder to get magnetite free of sulphate ions. This precipitate of magnetite was then dried at 100^0C and passed through sieve no.300.

8.6.2 Preparation of Gluteraldehyde saturated toluene (GST)[63]

Gluteraldehyde (100ml) and toluene (100ml) were placed in a beaker and stirred at 1000rpm for one hour using a magnetic stirrer. Then the solvent mixture was kept overnight for stabilization after which the upper toluene layer saturated with gluteraldehyde was decanted and used as gluteraldehyde saturated toluene (GST).

8.6.3 Preparation of Mesalamine Magnetic Microspheres[17,24,33]

Magnetic microspheres of Mesalamine was prepared by O/O solvent evaporation with chemical cross linking method. Accurately weighed quantity of polymer was dissolved in 10ml of 1% glacial acetic acid and accurately weighed drug was dissolved in minimum quantity of 0.1N HCl and added into the polymer solution.10mg of magnesium stearate was then added to the polymer-drug solution. Finally, specified amount of magnetite was added to this solution. The organic phase was drop-wise to 30ml of liquid paraffin containing 2% Span 80 and stirred at a speed of 1500rpm at 80^0C using high speed homogenizer. Stirring was continued for 1 h after the complete addition of polymer drug solution into oil. After 1 h stirring, 1-2 ml of GST was added dropwise to the mixture with continuous stirring at 500 rpm for the next 1 h at a temperature 50-55^0 C. Stirring was stopped after 1 h of addition of GST. Suspension of microspheres in paraffin oil thus obtained was centrifuged and the clear supernatant was decanted. Microspheres were then filtered and washed 3 times with hexane to remove liquid paraffin and then with distilled water to remove unentrapped drug from the surface of the microsphere. After that the microspheres were air dried and stored in dessicator at room temperature.

Table 11: Formulation of Mesalamine Magnetic Microspheres

Formulation code	Drug (mg)	Polymer (mg)		Magnetite (mg)	Magnesium Stearate 5% (mg)	Liq. paraffin (ml)	Span 80 (ml)	Drug: Polymer ratio
		Chitosan	Pectin					
F1	125	125	-	50	10	30	0.6	1:1
F2	84	166	-	50	10	30	0.6	1:2
F3	63	187	-	50	10	30	0.6	1:3
P1	125	-	125	50	10	30	0.6	1:1
P2	84	-	166	50	10	30	0.6	1:2
P3	63	-	187	50	10	30	0.6	1:3
Q1	125	62.5	62.5	50	10	30	0.6	1:1
Q2	84	42	83	50	10	30	0.6	1:2
Q3	63	31	94	50	10	30	0.6	1:3

8.7 EVALUATION OF MAGNETIC MICROSPHERES[68]

8.7.1 PHYSICOCHEMICAL CHARACTERIZATION[33]

8.7.1.1 Shape and surface morphology studies using Scanning Electron Microscope[33]

The shape and surface morphology of magnetic microspheres were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope.

8.7.1.2 Particle Size Analysis[24,38,60]

The average particle size of the prepared mesalamine magnetic microspheres for intra-arterial administration was measured by optical microscopy using a calibrated Eye piece micrometer and was compared to that of microspheres without drug and also with those microspheres prepared without drug and magnetite. The average size of 100 particles was determined by the equation.

Size of individual particle (μm) = Number of divisions on eye piece × Calibration factor

$$\text{Average Particle Size } (\mu m) = \frac{\text{Sum of Size of Individual Particles}}{\text{Number of particles}}$$

8.7.1.3 Drug- Excipient Interaction[24,34]

The FT-IR spectrum was recorded on Shimadzu FT-IR spectrophotometer, for the prepared mesalamine magnetic microspheres. The samples were prepared by grinding samples (5mg) with KBr (100mg) and then pressing the mixtures into pellets, further placed on a crystal sample holder and scanned from 4000cm^{-1} to 400cm^{-1}.

8.7.2 PHARMACEUTICAL CHARACTERIZATION

8.7.2.1 Percentage Yield[38]

Microspheres were weighed and the percentage yield was calculated by taking into consideration the total weight of the drug and excipients used for preparation of microspheres.

$$\text{Percentage Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \text{ X } 100$$

8.7.2.2 Estimation of Drug Content and Entrapment Efficiency[24,28,33]

50mg of microspheres was weighed and dissolved in 2.5ml of 0.1N HCl and suitably diluted with phosphate buffer saline pH 7.4 in 50 ml standard flask. The solution was kept for 24hrs and filtered to separate the fragments. Drug content was analyzed after suitable dilution by UV spectrophotometer at a wavelength of 230 nm against phosphate buffer saline pH 7.4 as blank. The drug content of each formulation was calculated using the following equation

$$\text{Percentage Drug Entrapment Efficiency} = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \text{ X } 100$$

8.7.2.3 Drug Loading Capacity[100]

Drug loaded microspheres were mixed in 2.5ml of 0.1N HCl and suitably diluted with phosphate buffer pH 7.4 at room temperature and kept for 24 h. After filtration and suitable dilution, Mesalamine present in the solution was determined.

$$\% \text{ Drug Loading} = \frac{\text{Quantity of the drug present in the microspheres}}{\text{Weighed quantity of microspheres}} \text{ X } 100$$

8.7.2.4 Swelling studies[33,38,63]

The degree of swelling of the microspheres was investigated in PBS pH 7.4 without enzymes. A dialysis membrane 9 cm long was activated by immersion in 50ml of distilled water regulated at 90^0C for 1 h and then washed with distilled water. A known weight of microspheres was placed in the activated dialysis membrane which was tied at both ends and immersed in a beaker placed on a thermostated bath maintained at 37^0C. At fixed intervals,

the membrane was removed from each medium, dried with filter paper and weighed. The degree of swelling (H) was calculated using the formula:

$$H = \frac{\text{Final weight of microspheres- Initial weight of microspheres}}{\text{Initial weight of microspheres}}$$

8.7.2.5 EVALUATION OF FLOW PROPERTIES[18,38,39,100]

Flow properties of microspheres were investigated by determining the following standard procedures.

8.7.2.5.1 Bulk Density

Bulk density was determined taking a known weight of dried microspheres in a measuring cylinder and tapping 3 times from 1 inch height at 2 second interval. The bulk volume is noted and the bulk density was calculated from the following equation.

$$\text{Bulk Density} = \frac{\text{Weight of microspheres}}{\text{Bulk volume of microspheres}}$$

8.7.2.5.2 Tapped Density

Tapped density is the ratio of mass of microspheres to the volume occupied by the same mass of the powder after a standard tapping of a measure. Weighed quantity of microspheres was taken in a cylinder and tapping 300 times from 1 inch at 2 second interval. The tapped volume is noted and the tapped density was calculated from the following equation.

$$\text{Tapped Density} = \frac{\text{Weight of microspheres}}{\text{Tapped volume of microspheres}}$$

8.7.2.5.3 Hausner's Ratio

Hausner's ratio is used for predicting the flow characteristics.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

8.7.2.5.4 Compressibility Index

Compressibility index was determined using bulk density and tapped density.

$$\text{Compressibility index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

8.7.2.5.5 Angle of Repose

A funnel was fixed to a stand and the bottom of the funnel was fixed at a height of 3 cm from the plane. Microspheres were placed in funnel and allowed to flow freely and the height and radius of the heap of microspheres was measured.

$$\tan \theta = h/r$$

Where, 'h' is the height of heap and

'r' is the radius of heap of microspheres

Table 12: Standards for Pre-compression Characteristics

Compressibility Index (%)	Hausner's Ratio	Angle of Repose (degrees)	Type of flow
<10	1.00-1.11	25-30	Excellent
11-15	1.12-1.18	31-35	Good
16-20	1.19-1.25	36-40	Fair
21-25	1.26-1.34	41-45	Passable
26-31	1.35-1.45	46-55	Poor
32-37	1.46-1.59	56-65	Very poor
>38	>1.60	>66	Very very poor

8.7.2.6 *in- vitro* Drug Release Study[24,115]

The *in- vitro* drug release study was carried out in basket apparatus using a mixture of 45ml of 0.1N HCl and 855ml of PBS pH 7.4 as the dissolution medium maintained at $37^0C \pm 0.5^0C$. Weighed microspheres containing 50mg of drug were introduced into the dissolution medium. Aliquots were taken at regular time intervals and after suitable dilution, percentage drug release analysed by UV Spectrophotometer at 230nm.

8.7.2.7 RELEASE KINETICS OF THE OPTIMIZED FORMULATION[37,45]

The *in- vitro* release data for the optimized batch was fitted to various release kinetic models (Zero-order, First- order, Higuchi, Hixon- Crowell and Korsmeyer- Peppas models). The goodness of fit was found out to describe the kinetics of drug release.

8.7.2.7.1 Zero order release model

Zero order models describe the systems where the drug release rate is independent of its concentration of the dissolved substance.

$$\mathbf{C = Ko\ t}$$

Where, C- Cumulative percentage drug released

Ko – zero-order constant

t- time

A plot of time on x- axis and cumulative percentage drug released on y-axis gives a straight line with slope, Ko if it follows zero-order Kinetics.

Application: This relationship can be used to describe the drug release of several types of modified release pharmaceutical dosage forms like trans-dermal systems, matrix tablets with low soluble drugs in coated forms and osmotic systems.

8.7.2.7.2 First order release model

First order models describe the systems where the release rate is dependent on the concentration of the dissolved substance.

$$\mathbf{Log\ C = log\ C_o - K\ t / 2.303}$$

Where, C – Cumulative percentage drug remaining

C_o- Initial concentration of drug

K – First order constant

A plot of time on x-axis and log cumulative percentage drug remaining on y – axis gives a straight line with slope, K / 2.303 if it follows first- order kinetics.

Application: This relationship can be used to describe the drug dissolution in pharmaceutical dosage forms containing water –soluble drugs in porous matrices.

8.7.2.7.3 Higuchi release model

The Higuchi model describes the release from systems where the solid drug is dispersed in an insoluble matrix and the rate of release is related to the rate of drug diffusion.

$$Q = K\sqrt{t}$$

Where, Q – Cumulative percentage drug released

K- Constant reflecting the design variables of the system

t – Time

A plot of square root of time on x-axis and cumulative percentage drug released on y-axis gives a straight line if it follows Higuchi Kinetics.

Application: This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms like some trans-dermal systems and matrix tablets with water soluble drugs.

8.7.2.7.4 Hixson-Crowell release model

The Hixson-Crowel cube root model describes the release from systems where there is a change in surface area and diameter of the tablets or particles.

$$Q_o^{1/3} - Qt^{1/3} = K_{HC}\, K\, t$$

Where, Qt – Cumulative percentage drug released in time t

Q_o – initial amount of the drug

K_{HC} – the rate constant for Hixson-Crowell rate equation

K – Constant incorporating the surface volume relation

A plot of time on x-axis and cube root of cumulative percentage of drug remaining on y-axis gives a straight line if it follows Hixson-Crowell kinetics.

Application: This equation applies to dosage forms like tablets, where the dissolution occurs in planes that are parallel to the drug surface if the tablet dimensions diminish proportionally, in such a manner that the initial geometrical form keeps constant.

8.7.2.7.5 Korsmeyer and Peppas Model:

Korsmeyer and Peppas Model derive a simple relationship which describes the drug release from a polymeric system.

$$M_t / M_\alpha = K t^n$$

Where, M_t / M_α – fraction of drug released at time t

K - Release rate constant

n - Release exponent

A plot of log time on x-axis and log cumulative percentage of drug released on y-axis gives a straight line, if it follows Korsmeyer and Peppas kinetics.

DIFFUSION COEFFICIENT	OVERALL SOLUTE RELEASE MECHANISM	EFFECT ON DRUG[11] RELEASE
0.45	Fickian diffusion	Only due to diffusion through the matrix
0.45<n<0.89	Anamolous (non-fickian diffusion)	Drug diffusion and polymer relaxation (erosion)
0.89	Case-II transport	Only due to polymer relaxation (erosion)
n>0.89	Super case-II transport	

In-vitro drug release data were fitted to various models such as zero- order, first-order, Higuchi equation, Korsmeyer- Peppas equation, and Hixson-Crowell equation to know about the mechanism of drug release:

1. C versus t (zero order)
2. log C versus t (first order)
3. Q versus square root of t (Higuchi)
4. Q_t versus cube root of t (Hixson-Crowell)
5. log% Q_t versus log t (Korsmeyer- Peppas)

8.7.2.8 Stability Studies[28,45,108]

Of the prepared microspheres, the formulation with optimum percentage drug release was selected for stability studies. The optimized formulation was placed separately in borosilicate screw capped glass containers in Stability chamber at a temperature of 40±2°C / 75±5% RH (climatic zone IV conditions for accelerated testing) and at ambient room temperature and humidity for a period of 60 days. The samples were assayed for drug content at regular intervals of 15th, 30th and 60th day.

8.7.3 PRECLINICAL *in-vitro* SCREENING STUDIES

8.7.3.1 *in- vitro* Drug Release Study in Microflora Activated System [89,96,98,102.]

Identification of an enzyme system that can simulate polysaccharide degradation in the colonic environment would be beneficial for a more mechanistic investigation. Commercially available ß-glucosidase preparation has the ability to degrade chitosan due to an existing chitinase in the enzyme. Similarly commercially available pectinase enzyme preparation has the potential to degrade pectin polymer. Therefore, such a mixture of enzyme preparation was used as one of the release media for the evaluation of *in- vitro* drug release study of optimised formulation .This study aims in investigating the possibility of its use as an *in-vitro* enzyme system to substitute for rat cecal and colonic enzymes and thereby limiting animal use in the initial screening study for such polymer-based colon delivery systems.

8.7.3.2 *in- vitro* Anti-inflammatory activity screening[85,93]

in- vitro anti-inflammatory activity screening has been carried out for optimized formulation as a pre stage for *in-vivo* animal studies

Inhibition of albumin denaturation

1 ml sample solution was withdrawn every one hour during *in- vitro* drug release study and thereafter, subjected to *in- vitro* anti-inflammatory analysis. Equal volume of distilled water was used for the purpose of control. To each reaction mixture, 1 ml of bovine albumin (1% in distilled water) was transferred and pH was adjusted to 6.3 by using small amount of 1 N HCl, Samples were incubated for30 min at 37^0 C in the dark followed by incubation at 57^0C for 5 min. Reaction tubes were then cooled under running tap water and turbidity of all the samples were recorded spectrophotometrically at 660 nm. Percentage inhibition of albumin denaturation was calculated by using the formula:

$$\text{Percentage inhibition} = \frac{\text{Abs Control- Abs Sample}}{\text{Abs Control}} \text{ X } 100$$

8.7.4 MAGNETIC CHARACTERIZATION

8.7.4.1.1 Preparation of 0.1N Sodium Thiosulphate[32]

Dissolve 25 g of sodium thiosulphate and 0.2 g of sodium carbonate in carbondioxide-free water and dilute to 1000ml with the same solvent.

8.7.4.1.2 Standardization of 0.1N Sodium Thiosulphate[32]

Dissolve 0.200 g of potassium bromate, weighed accurately, in sufficient water to produce 250 ml. To 50 ml of this solution add 2 g of potassium iodide and 3 ml of 2 M HCl and titrate with the sodium thiosulphate solution using starch solution, added towards the end of titration, as indicator until the blue colour is discharged.

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.002738 g of $KBrO_3$.

8.7.4.1.3 Determination of magnetite content[28,24,78,38]

Magnetite(Fe_3O_4) content in the prepared magnetically responsive microspheres was determined by the conventional titrimetric method using thiosulphate and potassium iodide for quantitative analysis. An accurately weighed amount of magnetic microspheres (after destruction by gentle heating) was dissolved in mixture of water (200ml) and conc. HCl (200ml) by heating it to the boiling point. After boiling for 15s, the solution was cooled rapidly. Then 3 g of potassium iodide was added and kept in dark for 15min, the liberated iodine was then titrated with 0.1 N sodium thiosulphate using starch as indicator. Similarly a blank titration was carried out. The difference between titrations gave the amount of iodine liberated by ferric ion.

Each ml of 0.1 N sodium thiosulphate is equivalent to 0.005585 g of ferric ion.

$$\text{Percentage Magnetite Content} = \frac{\text{(Blank- Titre value) x Equivalent factor x (Estimated molarity/ Expected molarity)}}{\text{Weight taken (equivalent to magnetite)}} \times 100$$

8.7.4.2 Magnetic Separation Behaviour [42]

The formulated magnetic microspheres were dispersed in water to study their magnetic separation behavior. The magnetic microspheres were made to move towards the direction of magnetic field in an aqueous solution under the externally applied magnetic field in 30s. After the ceassation of external magnetic field, the microspheres were checked for even spread out in water again with bottle shaking.

8.7.4.3 Magnetism Assessment using Vibrating Sample Magnetometer (VSM)[29,32,42]

Magnetic properties of microspheres containing magnetite were characterized via vibrating sample magnetometry. The VSM operates on the principle that when a sample material is placed in a uniform magnetic field, a dipole moment proportional to the product of the sample susceptibility times the applied field is induced in the sample. The sample also undergoing sinusoidal motion induces an electrical signal which is proportional to the magnetic moment, vibration amplitude and vibration frequency.

In single domain materials there is little or no hysteresis and the magnetic particles reach saturation faster compared to a multi domain material. If the sample contains multiple domains, a hysteresis loop in the magnetization curve is observed. This hysteresis from multi domain formation would cause a decrease in the response of the system.[30]

The magnetization curves of the magnetically responsive microspheres were taken at room temperature using Vibrating Sample Magnetometer (VSM) to study the super-para magnetic behavior by finding the saturation magnetization value.

9. RESULTS AND DISCUSSION

9.1 PREFORMULATION STUDIES

9.1.1 Drug- Polymer Compatibility Studies using FTIR Spectroscopy[24,34]

The compatibility between drug and polymer was confirmed by using FTIR spectroscopy.

Infrared spectroscopic analysis for drug (Mesalamine), Polymers (Chitosan, Pectin),magnesium stearate and Drug-Polymer mixture were carried out.

The principal IR peaks of pure Mesalamine and polymers Chitosan and Pectin are shown in table 13-16 and figure 19-22.

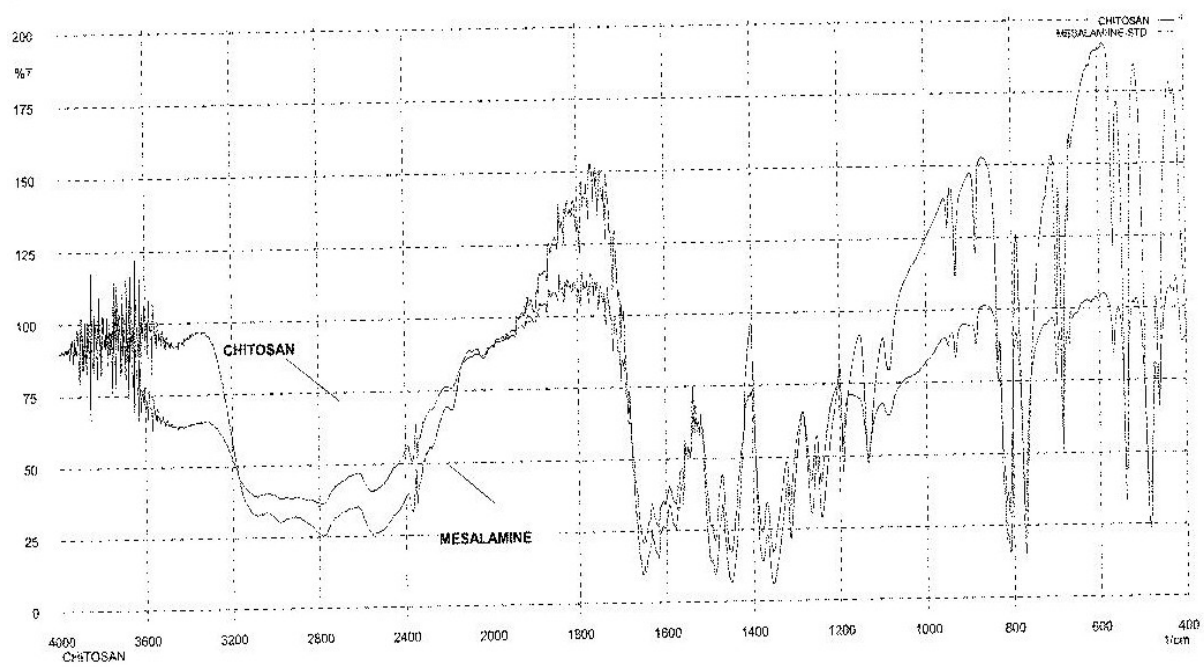


Figure 19: FTIR spectra of (Mesalamine + Chitosan) Vs. Mesalamine

Table 13: FTIR spectra of (Mesalamine + Chitosan) Vs. Mesalamine

Wave Number (cm^{-1})	Interpretation
2800-2900	O-H streching of carboxylic acid
3000-3050	Ar-H stretching
1550-1600	C=C stretching
3600-3700	Phenolic O-H stretching
1680-1700	C=O stretching of carboxylic acid
3300-3450	N-H stretching

Inference:

The FTIR study revealed that there is no interaction between the drug and polymer, since the major peaks of the drugs are not affected by the excipients

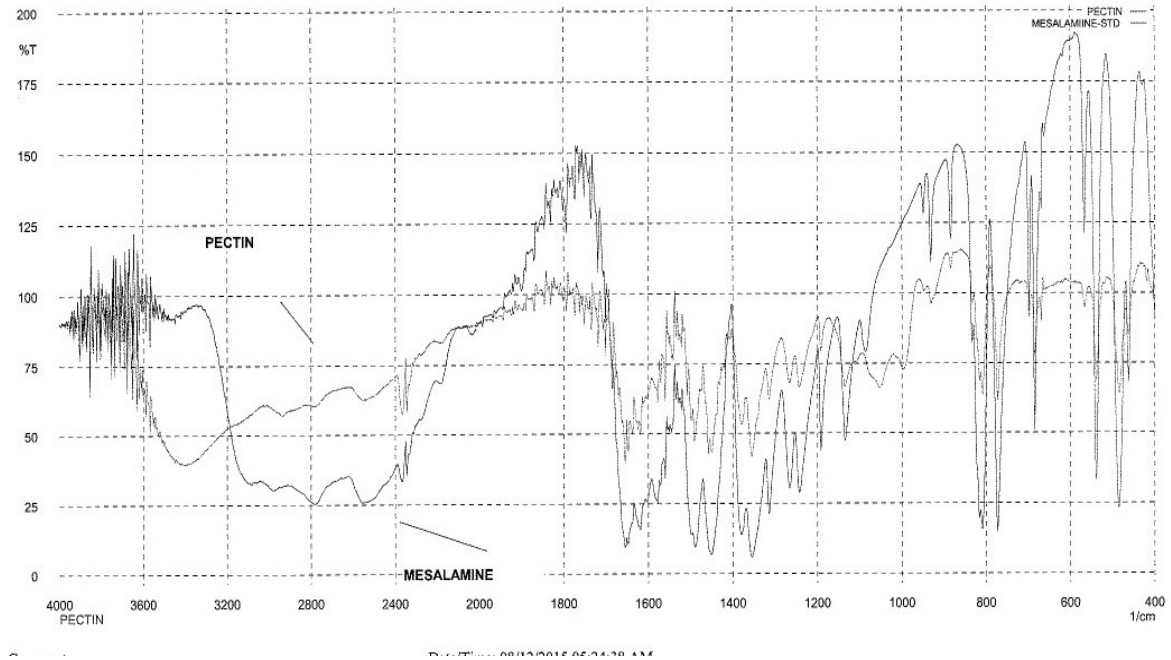


Figure 20: FTIR spectra of (Mesalamine + Pectin) Vs. Mesalamine

Table 14: FTIR spectra of(Mesalamine + Pectin) Vs. Mesalamine

Wave Number (cm^{-1})	Interpretation
2800-2950	O-H streching of carboxylic acid
3000-3050	Ar-H stretching
1550-1600	C=C stretching
3600-3700	Phenolic O-H streching
1680-1700	C=O stretching of carboxylic acid
3200-3400	N-H streching

Inference:

The FTIR study revealed that there is no interaction between the drug and polymer, since the major peaks of the drugs are not affected by the excipients

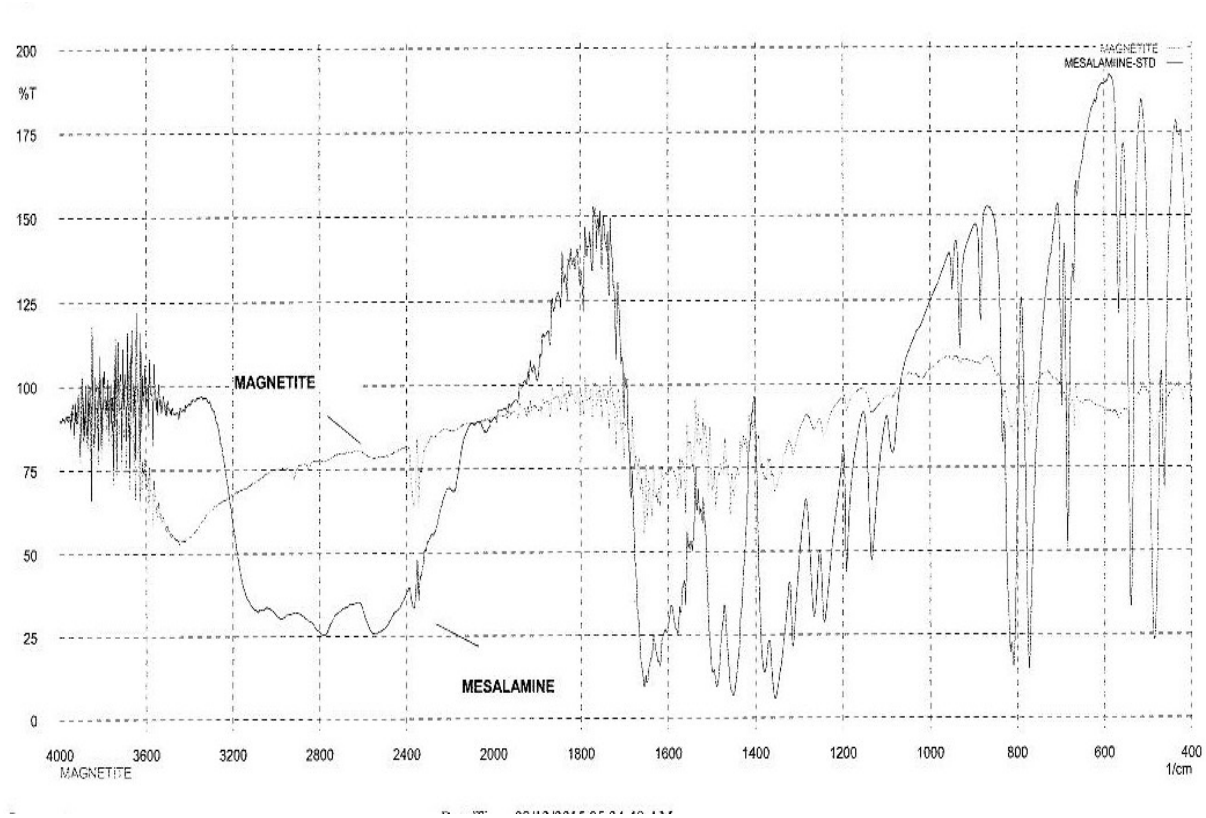


Figure: 21 FTIR spectra of (Mesalamine + Magnetite) Vs. Mesalamine

Table 15: FTIR spectra of (Mesalamine + Magnetite) Vs. Mesalamine

Wave Number (cm^{-1})	Interpretation
2900-2950	O-H streching of carboxylic acid
3000-3050	Ar-H stretching
1500-1600	C=C stretching
3500-3700	Phenolic O-H streching
1680-1700	C=O stretching of carboxylic acid
3200-3300	N-H streching

Inference:

The FTIR study revealed that there is no interaction between the drug and polymer, since the major peaks of the drugs are not affected by the excipients.

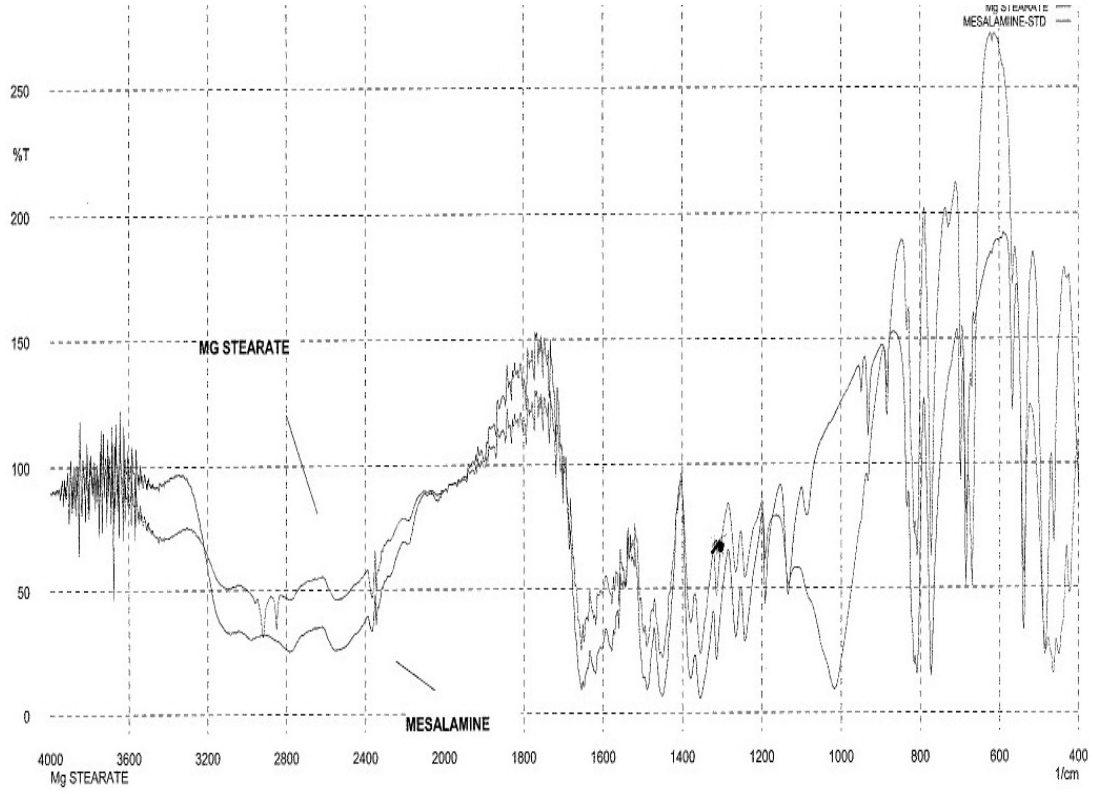


Figure: 22 FTIR spectra of(Mesalamine + Magnesium Stearate) Vs. Mesalamine

Table 16: FTIR spectra of (Mesalamine + Magnesium Stearate) Vs. Mesalamine

Wave Number (cm^{-1})	Interpretation
2800-2900	O-H streching of carboxylic acid
2950-3050	Ar-H stretching
1490-1550	C=C stretching
3500-3700	Phenolic O-H stretching
1680-1700	C=O stretching of carboxylic acid
3300-3400	N-H stretching

Inference:

The FTIR study revealed that there is no interaction between the drug and polymer. Hence it can be concluded that the major peaks of the drugs are not affected by the excipients. [29]

9.2 STANDARD CALIBRATION CURVE FOR MESALAMINE[32,53,86]

The UV Spectrophotometric method was used to analyze Mesalamine. The absorbance of the drug in phosphate buffer saline (pH 7.4) was measured at a wavelength of 230nm. The results are given in table 17 and figure 23.

Table: 17 Standard Curve for Mesalamine

S.No.	Concentration (µg/ml)	Absorbance (nm)
1.	0	0
2.	2	0.132±0.0043
3.	4	0.248± 0.0187
4.	6	0.375± 0.0533
5.	8	0.488 ±0.0305
6.	10	0.612± 0.0489

***Mean±SD(n=3)**

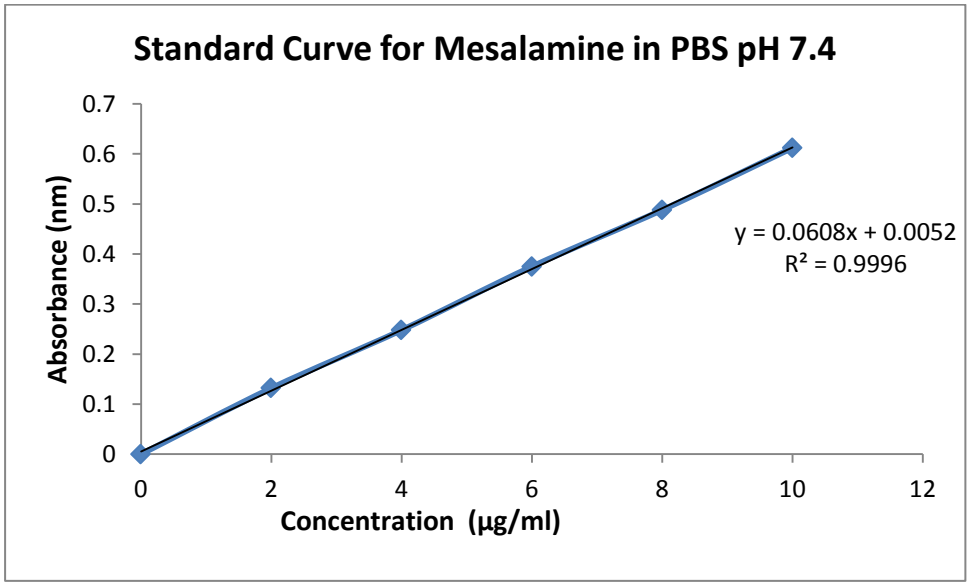


Figure 23: Standard Curve for Mesalamine in PBS pH 7.4

Inference

As shown in figure the linearity was exhibited at a concentration range of 0- 10 µg/ml of Mesalamine. It obeys Beer-Lambert's law.[53,86]

9.3 EVALUATION OF MICROSPHERES

9.3.1 PHYSICOCHEMICAL CHARACTERIZATION[33]

9.3.1.1 Scanning Electron Microscopy[33]

Morphological analysis of the microspheres was carried out using Scanning Electron Microscopy and the result is shown in figure 24.

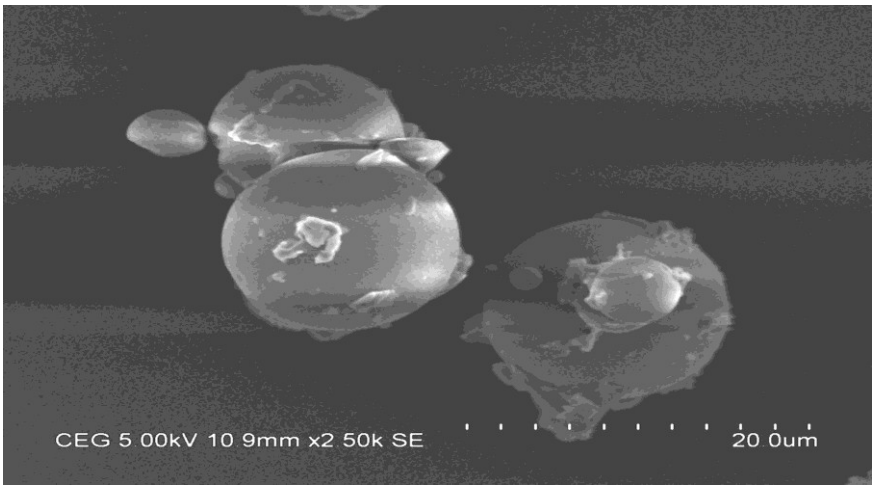


Figure 24: SEM Photographs of (a) Optimized formulation

Inference

The SEM photograph reveals that the microspheres were discrete and spherical in shape.

9.3.1.2 Mean Particle Size by Microscopy[24,38]

The particle size of various formulations of microsphere were determined by binocular microscopy. The results of mean particle size are depicted in the table 18 and figure 26.

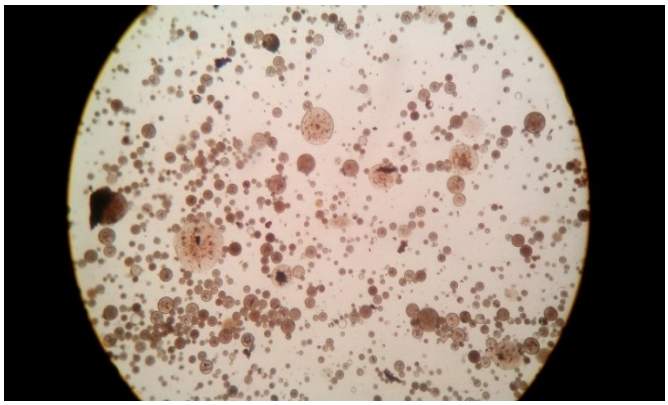


Figure 25: Photograph of Optimized formulation

Table 18: Mean Particle Size of Microspheres

Formulation code	Mean Particle Size(µm)
F1	34.95
F2	41.55
F3	41.70
P1	42.30
P2	45.15
P3	47.85
Q1	33.30
Q2	36.30
Q3	36.60

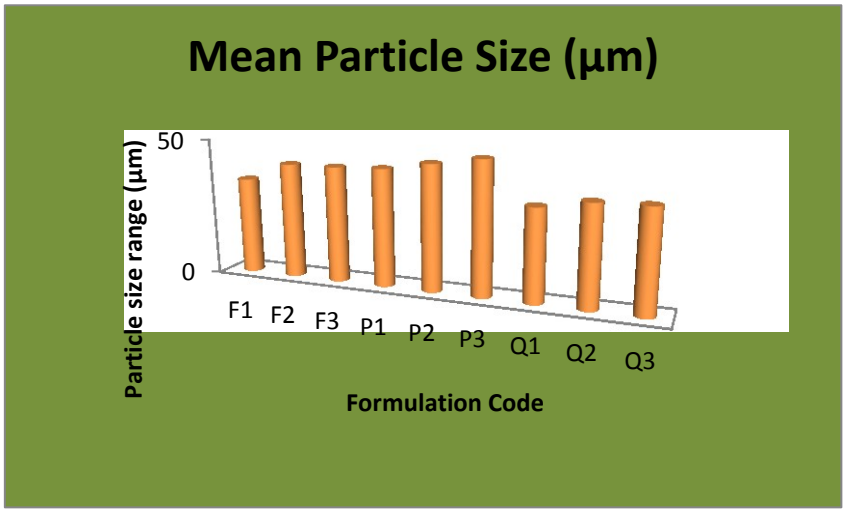


Figure 26: Mean Particle size of Microspheres

Inference

The data revealed that average particle size of microspheres increased with increasing polymer concentration. Higher concentration of polymer produced a more viscous dispersion with larger droplets and consequently larger microspheres were formed.[44,60,100]The particle size of microspheres was found in the range of 33.3 to 47.85 µm.

In general, less than 5µm size is used for intravenous route, less than 125 µm is used for intra-arterial route. Particles of this size can be administered easily by suspending them in a suitable vehicle and injecting them using a conventional syringe with an 18 or 20 gauge needle.[78]

9.3.1.3 Drug Excipient Interaction [24,34]

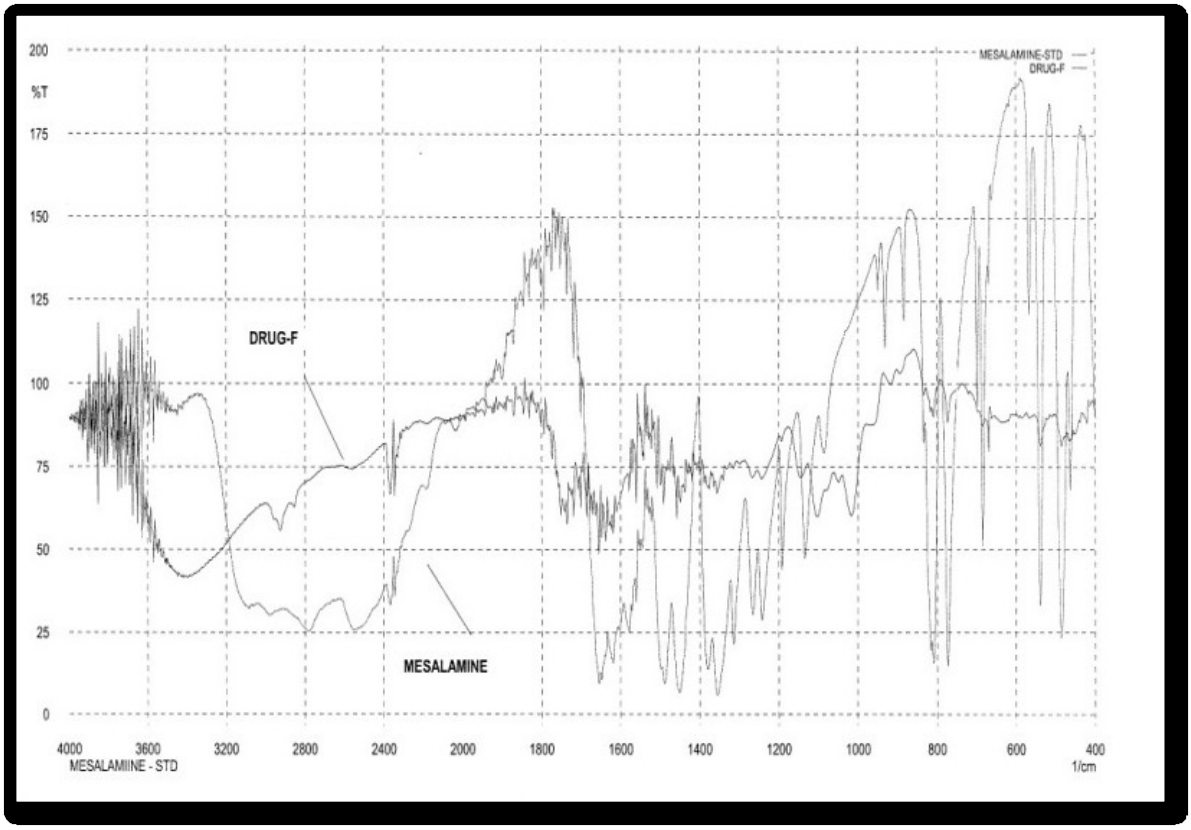


Fig 27: FT-IR Spectra of Optimized formulation

Table 19: FT-IR Spectra of Optimized formulation

Wave Number (cm^{-1})	Interpretation
2900-3000	O-H streching of carboxylic acid
2900-3000	Ar-H stretching
1550-1600	C=C stretching
3500-3700	Phenolic O-H stretching
1650-1750	C=O stretching of carboxylic acid

Inference:

The FTIR study revealed that there is no interaction between the drug and polymer in the optimized formulation Q1. Hence it can be concluded that the major peaks of the drug are not affected by the excipients. [24,34]

9.3.2 PHARMACEUTICAL EVALUATIONS

9.3.2.1 Percentage Yield[38]:

Percentage yield of various formulations are depicted in the table 20 and figure 28.

Table 20: Percentage yield

Formulation code	Theoretical yield (g)	Practical yield (g)	Percentage yield (% w/w)
F1	1.860	1.48	79.570
F2	1.240	0.973	78.468
F3	1.860	1.56	83.870
P1	1.860	1.688	90.753
P2	1.860	1.645	88.441
P3	1.860	1.570	84.409
Q1	1.860	1.723	92.634
Q2	1.860	1.680	90.323
Q3	1.860	1.500	80.645

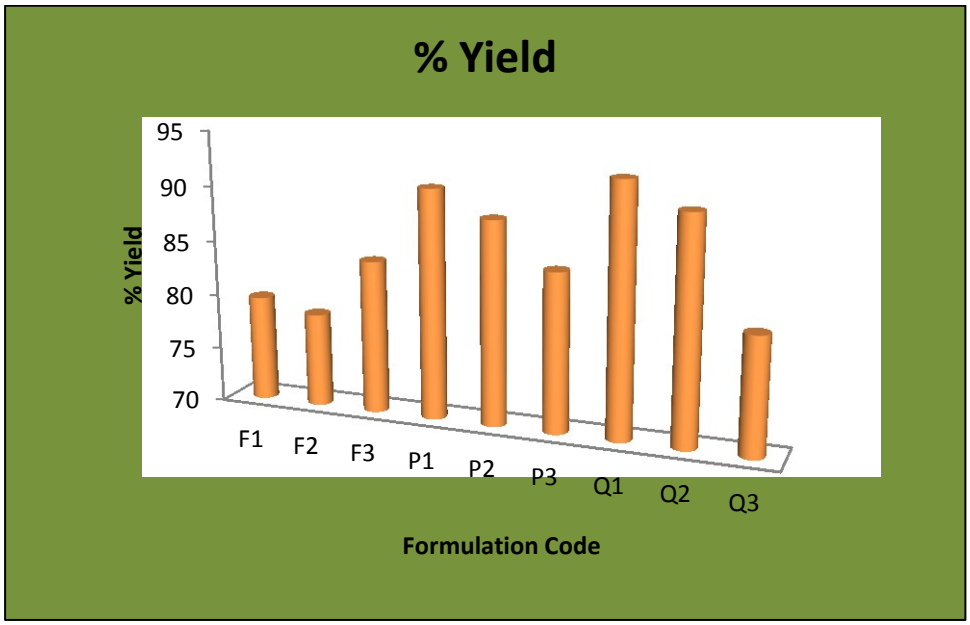


Figure 28: Percentage yield of Microspheres

Inference:

The percentage yield was found in the range of 79.57 to 92.634% w/w. The results indicated that the formulation containing chitosan-pectin combination (1: 1 ratio) yields better percentage of Mesalamine magnetic microspheres.

9.3.2.2 Drug Content and Entrapment Efficiency[24,28,33]

The content of active ingredients of various formulations was analyzed using UV spectrophotometer at 230 nm. The results of percentage drug content are depicted in table 21 and figure 29.

Table 21: Drug Content of Formulated Microspheres

Formulation code	Drug Content (%w/w)	SD (n = 3)
F1	85.058	1.68
F2	84.393	3.262
F3	72.157	5.42
P1	85.698	3.614
P2	78.127	0.24
P3	70.601	1.37
Q1	86.369	0.41
Q2	76.833	0.97
Q3	69.964	2.7

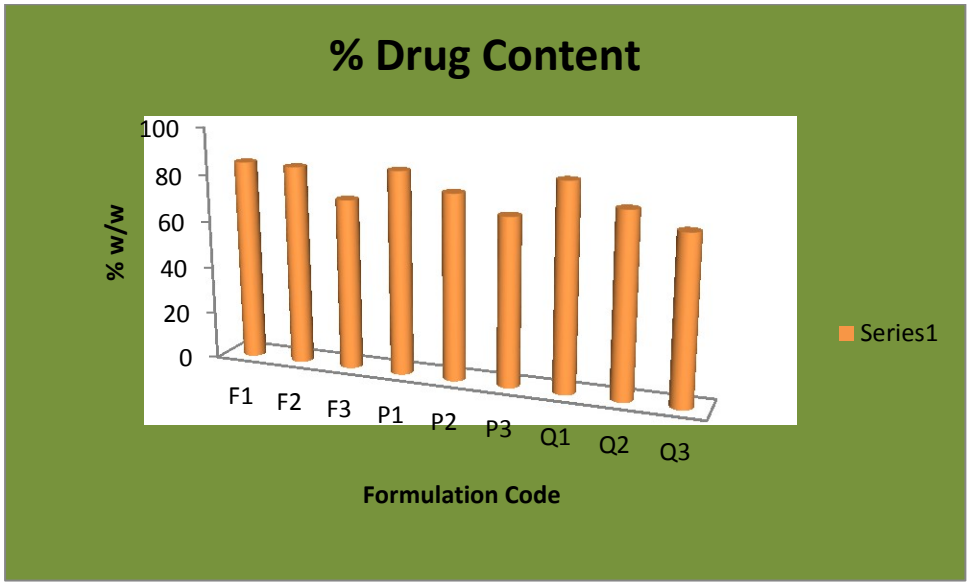


Figure 29: Drug content of Formulated Microspheres

Inference:

The percentage of drug content ranged from 69.964 to 86.369% w/w. The formulation Q1 found to have higher drug content.

9.3.2.3 Drug Loading Capacity[100]

The Drug loading capacity of various formulations was analyzed using UV spectrophotometer at 230 nm. The results of percentage drug content are depicted in table 22 and figure 30.

Table 22: Drug Loading Capacity of Formulated Microspheres

Formulation code	Drug Loading (%)	SD (n = 3)
F1	17.289	0.34
F2	22.828	0.672
F3	29.093	2.20
P1	19.131	0.302
P2	21.667	0.066
P3	19.131	0.37
Q1	17.540	0.059
Q2	20.819	0.26
Q3	28.209	1.09

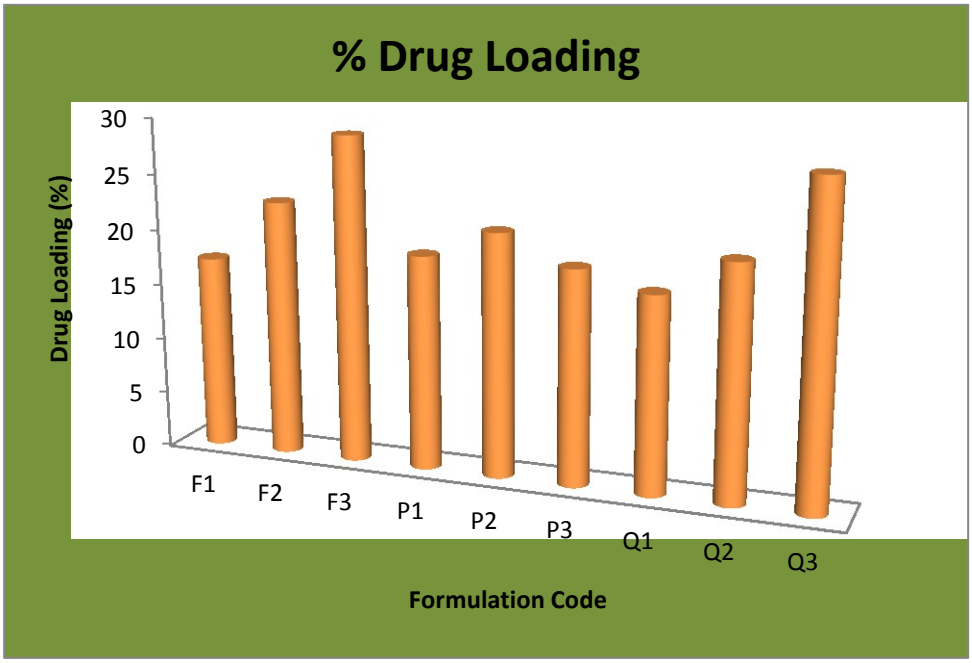


Figure: 30 Drug Loading of Formulated Microspheres

Inference

The drug loading capacity ranged from 17.289 – 28.209 %w/w and from the result it is clear that the drug loading capacity increases with increase in drug:polymer ratio.[25,101.]

9.3.2.4 Swelling Studies[33,38,63]

The swelling ability of various microsphere formulations in PBS pH 7.4 was determined and the results of their % swelling (% SR) was shown in table 23 and figure 31.

Table 23: % Swelling Vs. % Drug content

Formulation code	Swelling Ratio (SR)	Drug Content (%w/w)
F1	32.4	85.058
F2	36.0	84.393
F3	38.0	72.157
P1	20.1	85.698
P2	21.0	78.127
P3	21.5	70.601
Q1	20.0	86.369
Q2	26.0	76.833
Q3	33.0	69.964

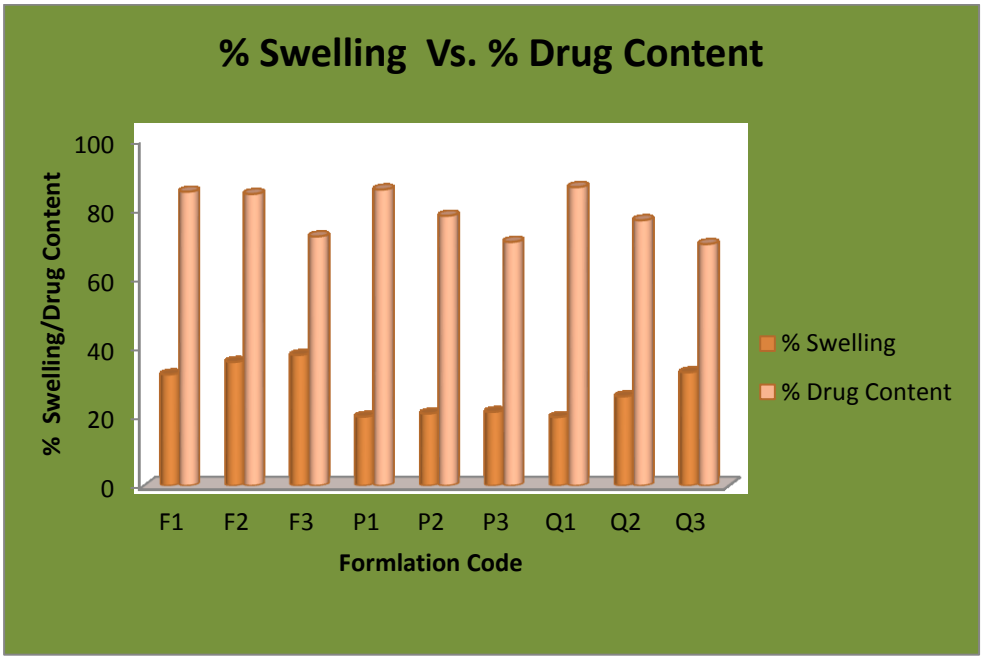


Figure 31: Percentage Swelling Vs. Percentage Drug content

Inference

The swelling ratio of microspheres was found in the range of 0.200 to 0.380. The data revealed that the SR of microspheres increases with decrease in drug content since water molecules cannot acquire much space in MM with higher drug entrapment.[33]

9.3.2.5 EVALUATION OF FLOW PROPERTIES[18,38,39,100]

Table 24: Flow Properties of Mesalamine Magnetic Microspheres

Formulation code	Bulk density(g/ml)	Tapped density(g/ml)	Compressibility index (%)	Hausner's ratio	Angle of repose (°)
F1	0.335± 0.013	0.427 ± 0.035	21.11 ± 4.189	1.270± 0.065	26.31± 2.257
F2	0.316 ± 0.008	0.366 ± 0.013	13.52± 1.753	1.156± 0.023	29.3± 1.892
F3	0.297 ±0.00	0.337 ± 0.007	12.04± 1.676	1.137± 0.024	27.281± 0.571
P1	0.467 ± 0.009	0.523± 0.017	10.203± 1.566	1.112± 0.023	29.55± 1.536
P2	0.385 ± 0.011	0.427± 0.009	7.550± 3.954	1.108± 0.028	27.36± 0.953
P3	0.33 ± 0.0	0.363± 0.005	6.38± 3.008	1.069± 0.036	28.16± 1.076
Q1	0.415 ± 0.0	0.448± 0.016	6.775± 1.994	1.07± 0.022	26.57± 0.844
Q2	0.416 ± 0.008	0.484± 0.017	13.808± 1.900	1.161± 0.025	30.28± 1.78
Q3	0.203 ± 0.011	0.517± 0.022	12.29± 4.174	1.142± 0.057	27.24± 1.045

***Mean±SD(n=3)**

9.3.2.5.1 Bulk Density

The results of bulk density measurements are shown in table 24 and figure 32.

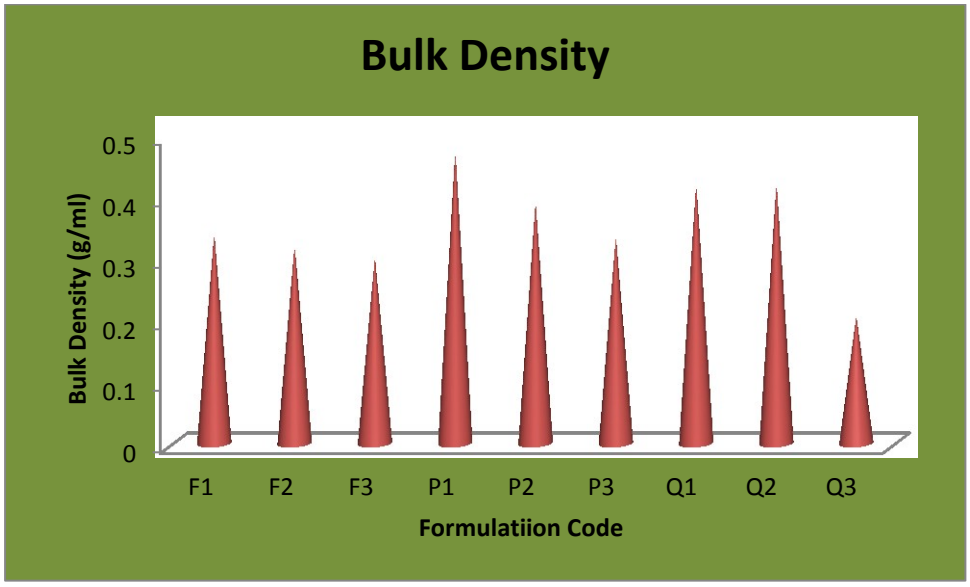


Figure 32: Bulk density of Microspheres

The bulk density was found in the range of 0.203 to 0.467 g/ml for various formulations.

9.3.2.5.2 Tapped density

The results of tapped density measurements are shown in table 24 and figure 33.

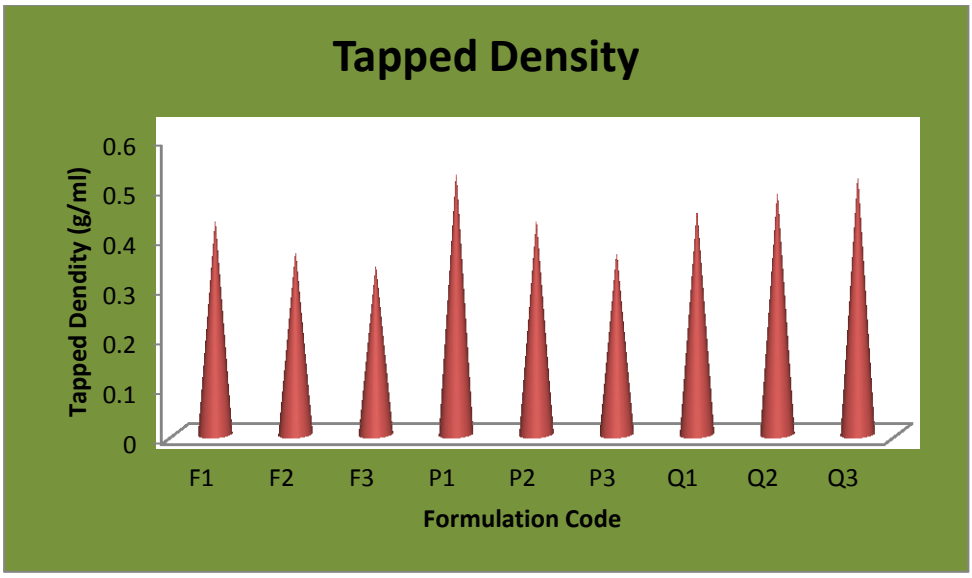


Figure 33: Tapped density

The tapped density was found in the range of 0.337 to 0.523 g/ml for various formulations.

9.3.2.5.3 Angle of repose

The results of angle of repose measurements are shown in table 24 and figure 34.

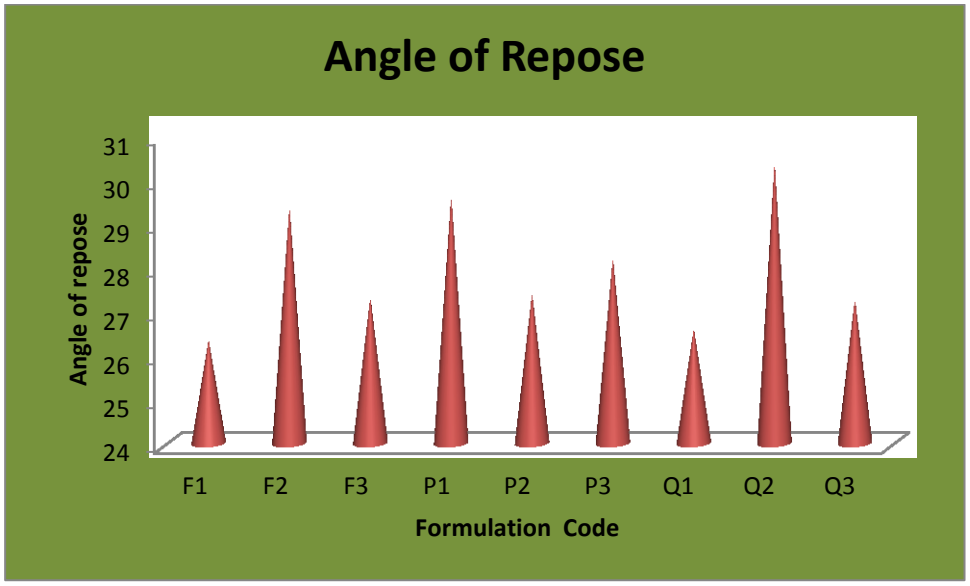


Figure 34: Angle of repose

The angle of repose of various formulations ranges from 26.31^{o} to 30.28^{o} indicating excellent flow of microspheres

9.3.2.5.4 Compressibility Index (%)

The results of compressibility index measurements are shown in table 24 and figure 35.

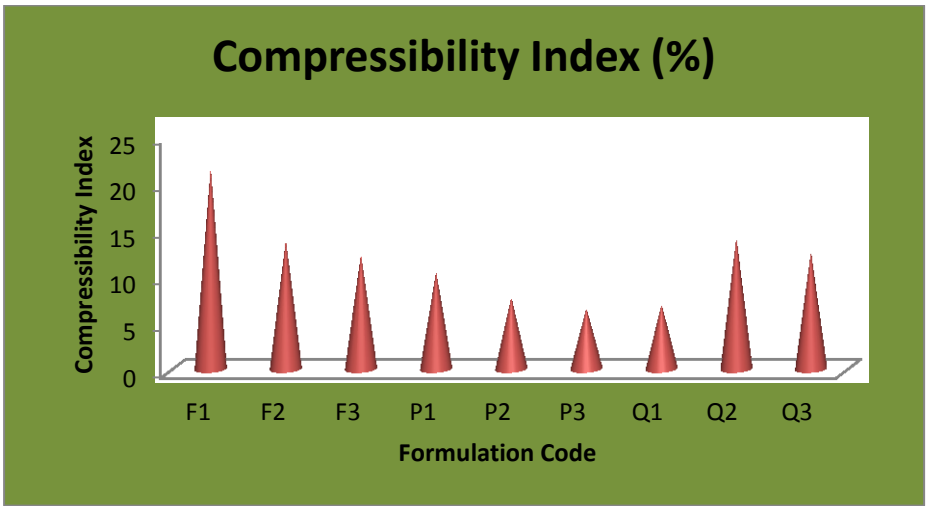


Figure 35: Compressibility index (%)

The compressibility index was found in the range of 6.38 to 21.11% for various formulations and shows excellent-passable flow properties.

9.3.2.5.5 Hausner's Ratio

The results of Hausner's Ratio measurements are shown in table 24 and figure 36.

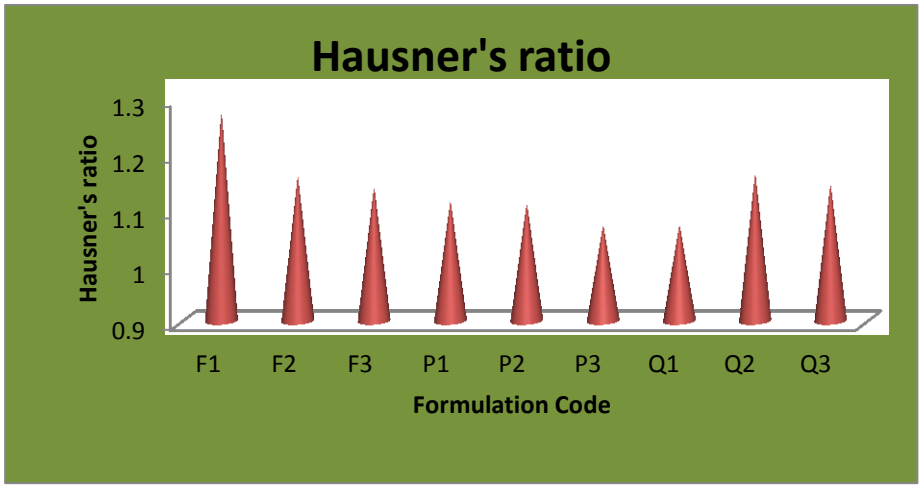


Figure 36: Hausner's Ratio

The Hausner's ratio of the formulations were found in the range of 1.07 to 1.161and shows excellent-good flow properties.

Inference

The flow properties of the microspheres were evaluated. It is found that the flow of microspheres are optimum and within the range.[18] It indicates good flow characteristics.

9.3.2.6 *in- vitro* RELEASE STUDIES[24,115]

The *in- vitro* release study of Mesalamine magnetic microspheres was done using basket apparatus using a mixture of 45ml of 0.1N HCl and 855ml of PBS pH 7.4 as the dissolution medium . The results are shown in table 25-27 and figure 37-39.

9.3.2.6.1 Table: 25 Percentage drug release of Pure Drug, Formulation F1 to F3

Time (hours)	Pure Drug	F1	F2	F3
0	0	0	0	0
0.5	29.160	-	-	-
1	93.400	4.464	3.960	7.200
1.5	110.48	-	-	-
2	-	9.024	10.092	10.480
3	-	15.916	11.240	19.348
4	-	21.404	18.860	25.212
5	-	48.520	24.364	45.504
6	-	63.908	47.180	68.788
7	-	89.820	81.998	99.400
8	-			106.060

***Mean±SD(n=3)**

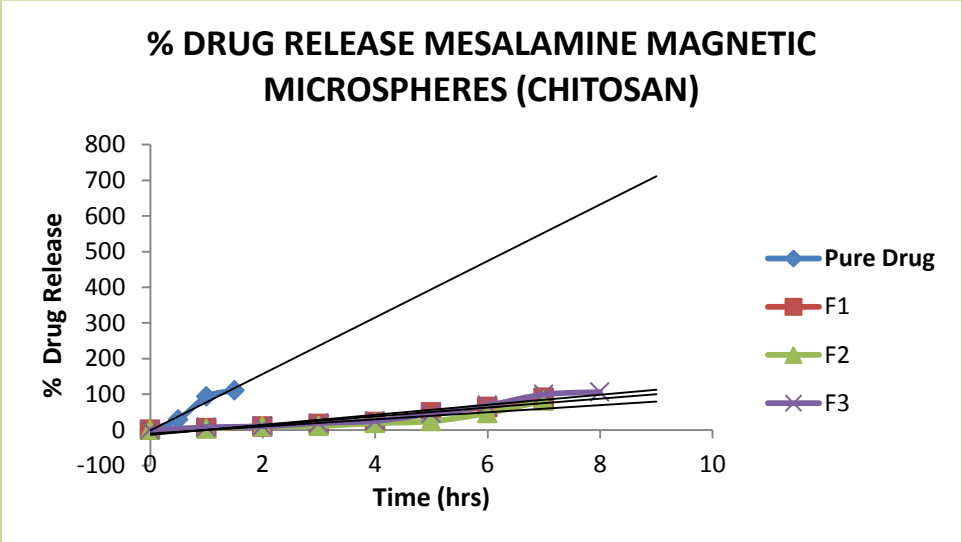


Figure 37: Percentage Drug release of Mesalamine Magnetic Microspheres (Chitosan)

It is observed that all the formulations gave the drug release less than 10±1% in first 2 hrs in PBS (pH 7.4) As drug: polymer ratio increases (i) drug release decreases due to the formation of a rigid polymer matrix and (ii) particle size increases, thus surface area is decreased and the drug release is retarded for the formulation F3 with the highest drug:polymer ratio (1:3).[13]

9.3.2.6.2 Table 26: Cumulative percentage drug release of Formulation P1 to P3

Time (hours)	Pure Drug	P1	P2	P3
0	0	0	0	0
0.5	29.160	-	-	-
1	93.400	46.800	43.200	28.8
1.5	110.48	-	-	-
2	-	55.700	53.520	40.20
3	-	64.288	58.856	46.68
4	-	107.482	98.060	80.056
5	-		-	100.296

***Mean±SD(n=3)**

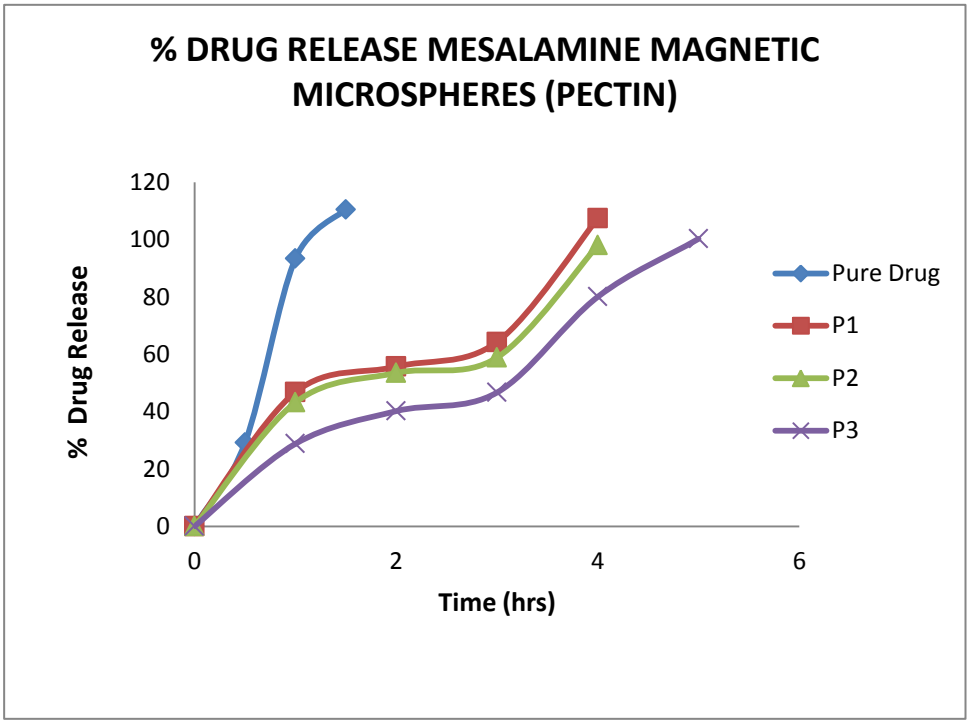


Figure 38: Percentage Drug release of Mesalamine Magnetic Microspheres (Pectin)

The results indicated that formulation with lesser drug–polymer ratio shows faster drug release. All formulations from P1 to P3 showed a burst release of 50±5% in 2h itself. The burst release may be due to high solubility of pectin.[115]

9.3.2.6.3 Table 27: Cumulative percentage drug release of Formulation Q1 to Q3

Time (hours)	Pure Drug	Q1	Q2	Q3
0	0	0	0	0
0.5	29.160	-	-	-
1	93.400	6.840± 0.294	15.840	10.44
1.5	110.48	-	-	-
2	-	14.078± 0.295	18.808	14.46
3	-	21.476± 0.452	21.600	19.218
4	-	30.808± 0.625	43.872	35.884
5	-	58.243± 0.349	64.994	79.282
6	-	76.534 ±0.449	89.832	90.520
7	-	95.137 ±0.162	104.006	103.618
8	-	99.019± 0.181	-	-
9	-	100.64 ±0.332	-	-

***Mean±SD(n=3)**

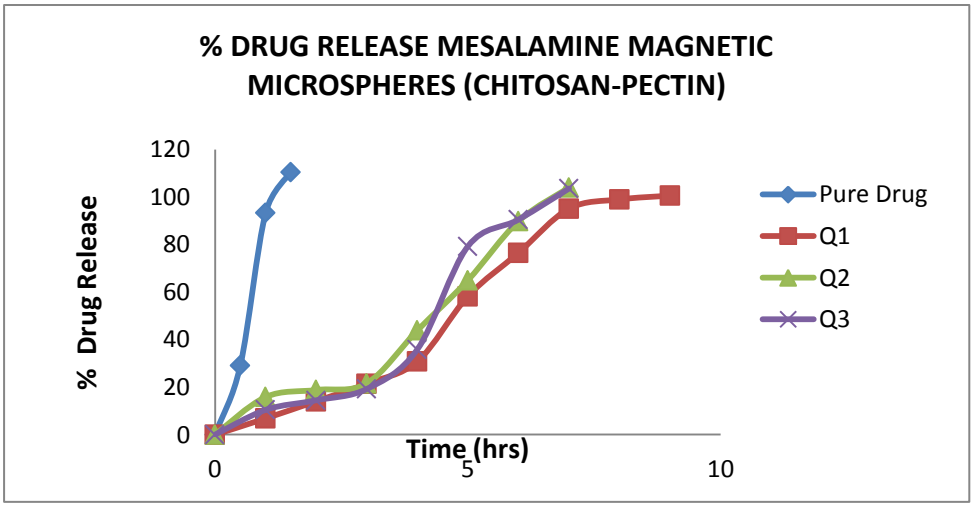


Figure 39: % Drug release of Mesalamine Microspheres (Chitosan-Pectin combination)

It is observed that the formulations Q1, Q2 and Q3 containing polymer mixture (chitosan and pectin in the ratios 1:1, 1:2, and 1:3 respectively) was able to protect the formulation from premature drug release when compared to those formulations containing pectin alone (namely, P1, P2 and P3).The results also indicates that the chitosan-pectin microspheres substantially retarded the drug release and showed the best result for the one with higher chitosan content (i.e., Q1 formulation). The inter polymer complex that could be formed between carboxyl groups of pectin and the amino groups of chitosan, may be responsible for such delayed drug release.[68]

9.3.2.6.4 EFFECT OF POLYMER TYPE ON *in- vitro* DRUG RELEASE[68]

The effect of polymer type on *in- vitro* drug release was compared. The results are shown in table 28 and figure 40.

Table 28: Percentage drug release of Formulations F1, P1 and Q1

Time (hours)	F1	P1	Q1
0	0	0	0
1	4.464	46.800	6.840
2	9.024	55.700	14.078
3	15.916	64.288	21.476
4	21.404	107.482	30.808
5	48.520	-	58.243
6	63.908	-	76.534
7	89.820	-	95.137
8	-	-	99.019
9	-	-	100.640

***Mean±SD(n=3)**

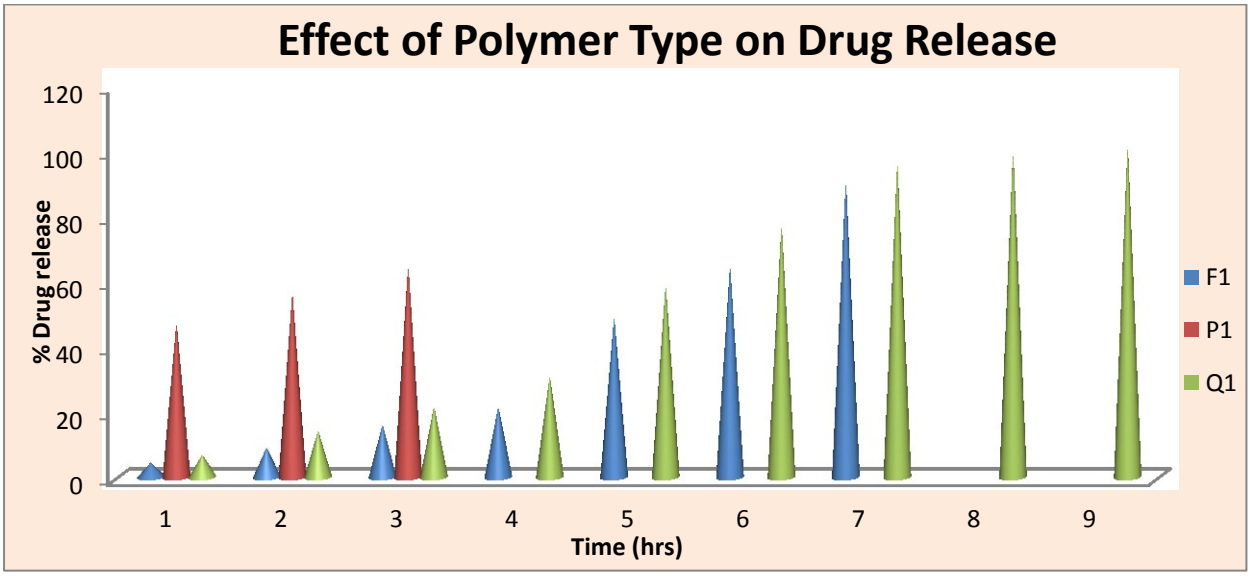


Figure 40: Percentage drug release of Formulations F1, P1 and Q1

It is observed that the formulation Q1 containing polymer mixture (chitosan and pectin in the ratio (1:1) was able to protect the formulation from premature drug release when compared to those formulation containing pectin alone (P1).The results also indicates that the chitosan-pectin microspheres substantially retarded the drug release upto 9 hours. The inter polymer complex that could be formed between carboxyl groups of pectin and the amino groups of chitosan, may be responsible for such delayed drug release.[68]

9.3.2.9 RELEASE KINETICS AND MECHANISM[37]

The kinetics of drug release for optimized Mesalamine loaded Chitosan-Pectin (1:1ratio) magnetic microspheres Q1 was shown in the table 29 and figure 41 -45.

Table 29: Mechanism of release kinetics

Time in Hours	% Cum. Drug release	% Cum. Drug remaining	Log% Cum. drug remaining	Square root of time	Log time	Log% cum. Drug release	Cube root of % drug remaining
0	0	100	2	0	-α	-α	4.642
1	6.84	93.16	1.969	1	0	0.835	4.533
2	14.078	85.922	1.934	1.41421	0.30103	1.148	4.413
3	21.476	75.524	1.878	1.73205	0.47712	1.332	4.227
4	30.808	69.192	1.84	2	0.60206	1.489	4.105
5	58.243	41.757	1.621	2.23607	0.69897	1.765	3.469
6	76.13	23.87	1.378	2.44949	0.77815	1.881	2.879
7	95.137	4.863	0.867	2.64575	0.8451	1.978	1.694
8	99.019	0.981	0.008	2.82843	0.90309	1.996	0.994

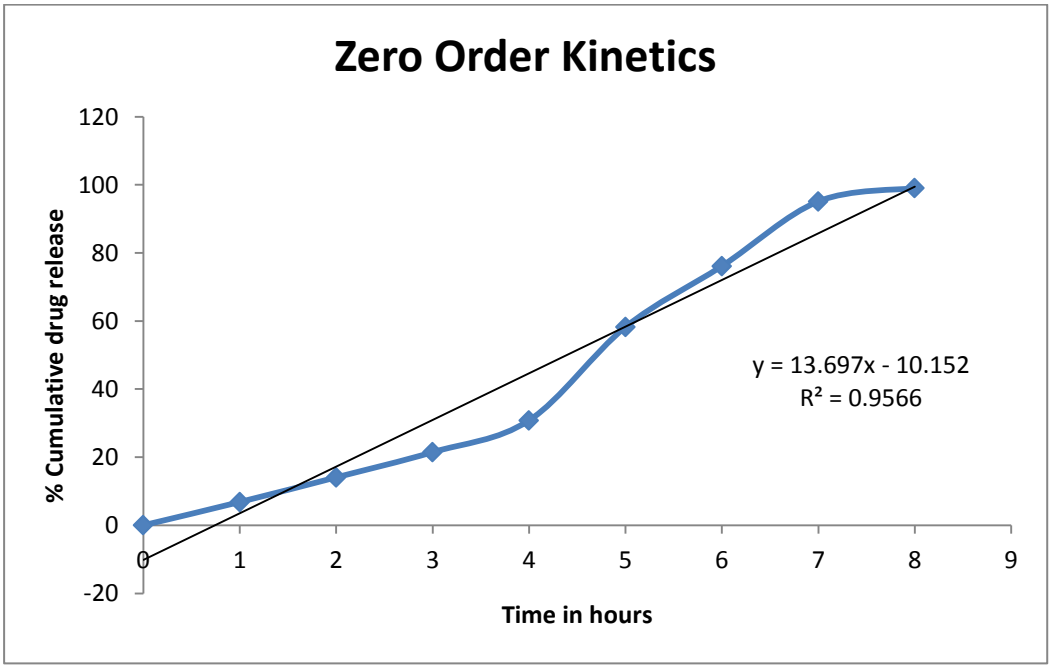


Figure 41: Zero Order Kinetics for Optimized Formulation Q1

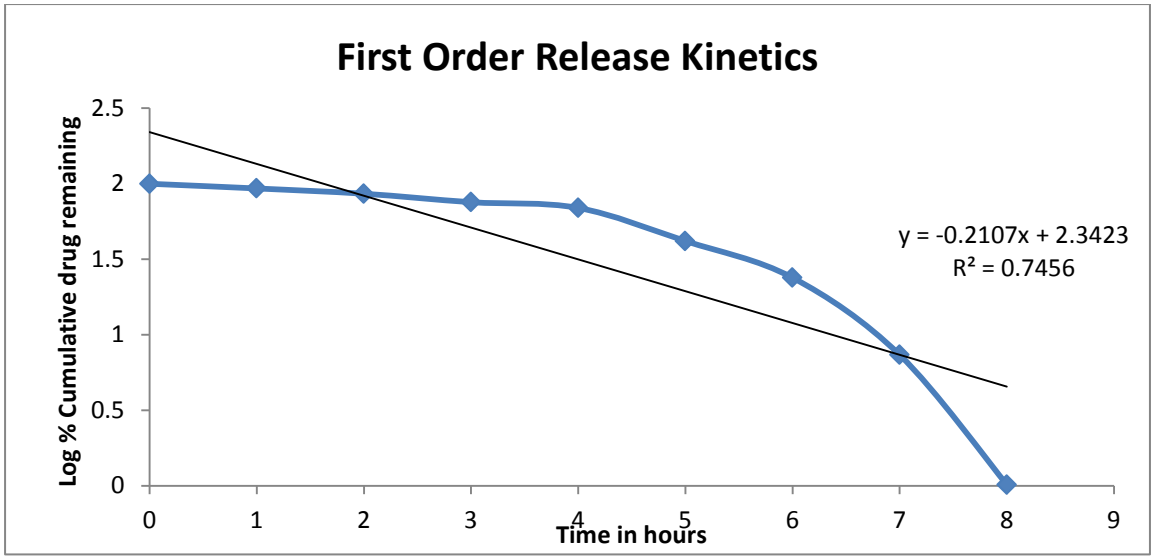


Figure 42: First Order Kinetics for Optimized Formulation Q1

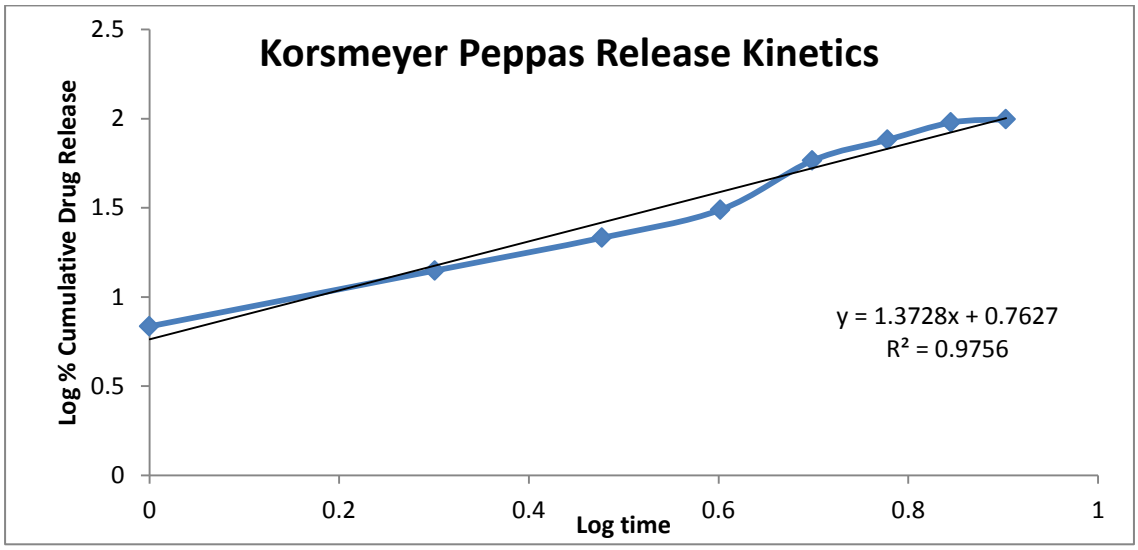


Figure 43: Korsmeyer Peppas Release Kinetics for Optimized Formulation Q1

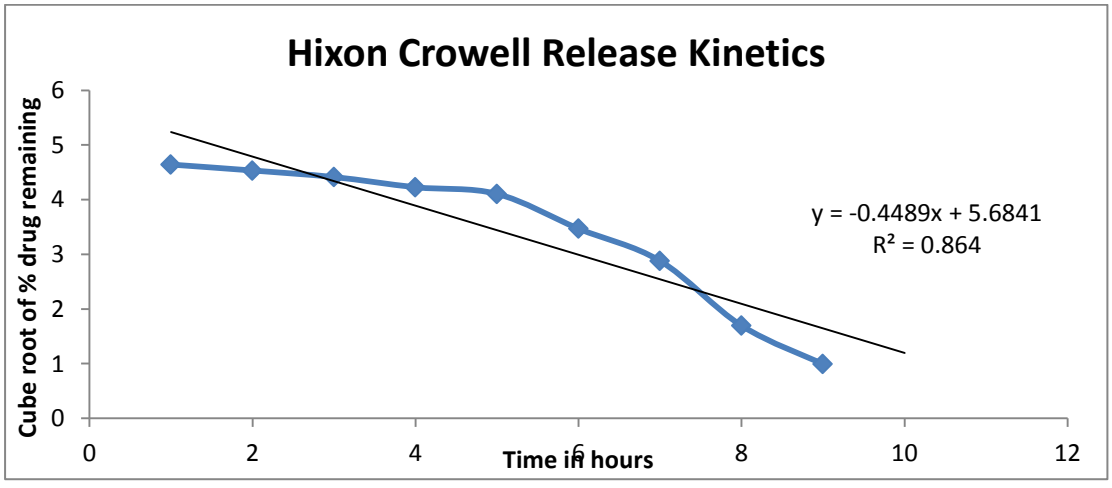


Figure 44: Hixon Crowell Kinetics for Optimized Formulation Q1

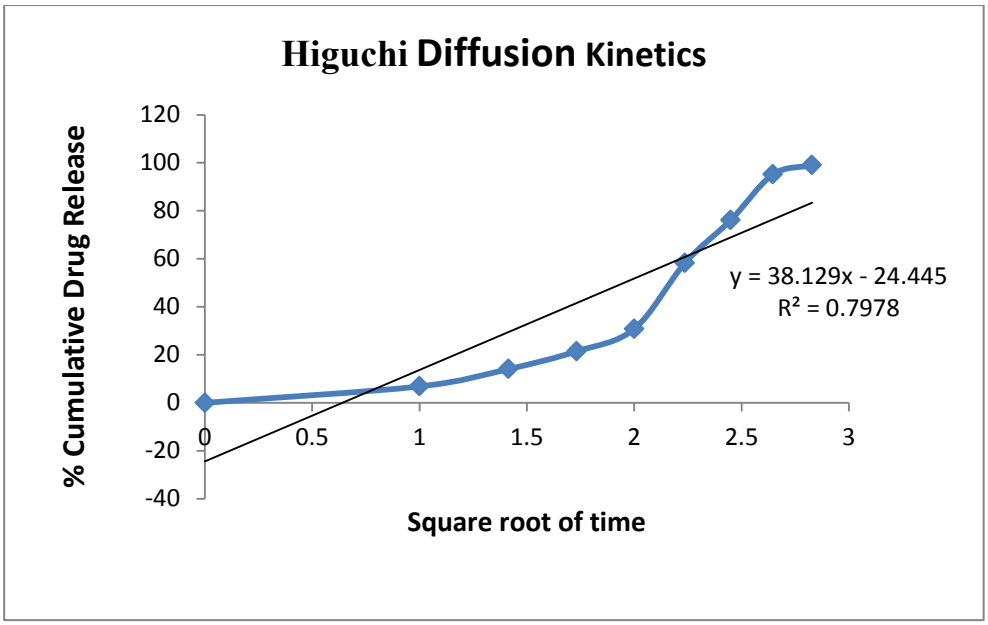


Figure 45: Higuchi Diffusion Kinetics for Optimized Formulation Q1

The coefficient of determination (R^2) was taken as criteria for choosing the most appropriate model. The R^2 values of various kinetic models are in table 30.

Table 29: R^2 values of various kinetic models

Kinetic model	Coefficient of determination (R^2)
Zero order	0.956
First order	0.745
Korsmeyerand Peppas	0.975
Hixson-Crowell	0.864
Higuchi	0.797

- The order of drug release was found to be Zero order, in which regression value was 0.956.
- The 'n' value of Korsmeyer peppas equation was found to be 1.372. From this it was concluded that the drug release follows a Super-Case II transport in which the drug release mechanism may be due to polymer relaxation (erosion) alone.[37,45,44]

9.3.2.10 STABILITY STUDIES [28,45,108]

During storage at different temperature and humidity conditions (room temperature as well as in stability chamber at 40±2°C/75±5% RH), the optimized formulation Q1 was assayed at regular intervals of 15th, 30th and 60th days. The results are shown in table 30 and figure 46.

Table 30: Stability Studies

Parameter	Initial		15th day		30th day		60th day	
	Ambient temperature & humidity	40±2°C / 75±5% RH	Ambient temperature & humidity	40±2°C / 75±5% RH	Ambient temperature & humidity	40±2°C / 75±5% RH	Ambient temperature & humidity	40±2°C / 75±5% RH
Physical appearance	No change	No change	No change	No change	No change	No change	No change	No change
Drug Content	86.365 ±0.41	86.365 ±0.41	85.945 ± 0.12	86.102 ±0.023	85.892 ±0.196	85.997 ±0.074	85.17 ±0.18	84.867 ± 0.07

*Mean±SD(n=3)

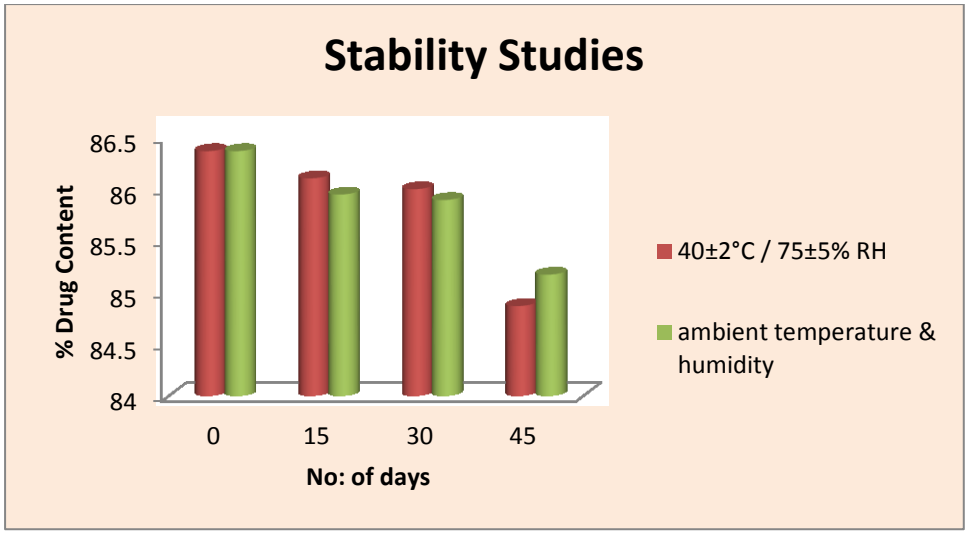


Figure 46: Stability Data of Optimized formulation

Inference

As per the stability data obtained, there is no significant change either in physical appearance or in drug content. Thus the formulation was stable at different conditions of temperature and humidity.[28,44]

Preclinical *in-vitro* Screening Studies

9.3.2.7 *in-vitro* DRUG RELEASE STUDY IN MICROFLORA ACTIVATED SYSTEM[98,102,96,89]

The effect of ß-glucosidase and pectinase enzymes on the drug release of optimized formulation Q1 is shown in the table 31 and figure 47.

Table 31: Cumulative percentage drug release of Q1 with & without Enzyme

Time (hours)	Cumulative % Release	
	Without Enzyme	**With ß-glucosidase & pectinase enzymes**
0	0	0
1	6.840± 0.294	9.720
2	14.078± 0.295	17.694
3	21.476± 0.452	36.152
4	30.808± 0.625	57.772
5	58.243± 0.349	83.004
6	76.534 ±0.449	97.45
7	95.137 ±0.162	106.144
8	99.019± 0.181	-
9	100.64 ±0.332	-

***Mean±SD(n=3)**

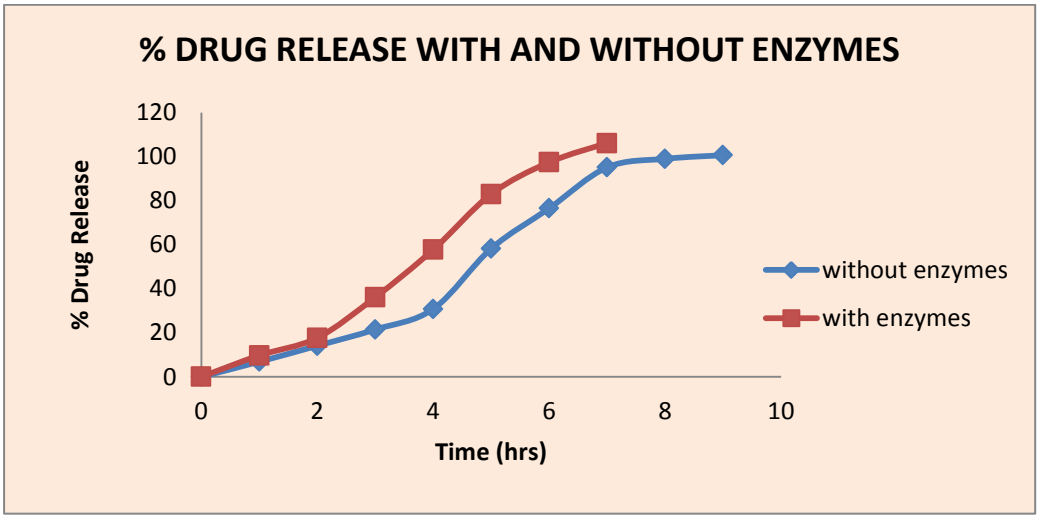


Figure 47: % Drug release of Optimized Formulation with & without enzymes

In the presence of enzymes (ß-glucosidase containing a chitosinase enzyme which can hydrolyze chitosan polymer and pectinase enzyme which can hydrolyze pectin polymer), the polysaccharides undergoes a faster erosion process resulting in the rapid dissolution in the medium ; eventually facilitating faster drug release with a maximum of 106.144% achieved within 7 hours.[37,70]

9.3.2.8 *in- vitro* ANTI-INFLAMMATORY ACTIVITY OF OPTIMIZED FORMULATION[85,93]

The *in- vitro* percentage inhibition of albumin denaturation method was used to evaluate anti-inflammatory potential of the formulation Q1 and the results are shown in the table32 and fig 48.

Table 32: Inhibition of protein denaturation assay of optimized formulation Q1

Time (hours)	% Inhibition	
	Pure Drug	**Formulation Q1**
0	0	0
0.5	39.858	-
1	80.527	18.450
1.5	82.657	-
2	-	24.544
3	-	28.701
4	-	41.886
5	-	63.692
6	-	73.120
7	-	80.730
8	-	81.237
9	-	82.252

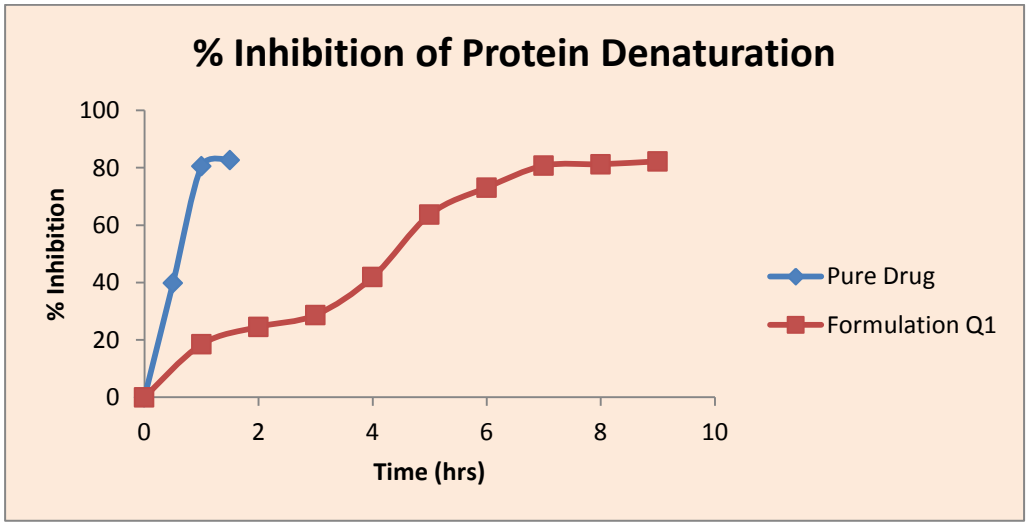


Figure 48: %Inhibition of Protein Denaturation of Optimized formulation Q1

Inference

The results shows that optimized formulation Q1 showed 82% of albumin denaturation within 9 h which clearly indicates that Q1 also exhibited a satisfactory dose-dependent anti-inflammatory activity as that of the pure drug.[93]

9.3.3 MAGNETIC CHARACTERIZATION[1]

9.3.3.1 Percentage Magnetite Content[28,24,78,38]

Magnetite (Fe_3O_4) content in the prepared magnetically responsive microspheres was determined by the conventional titrimetric method using thiosulphate and potassium iodide for quantitative analysis and the results are shown in table 33 and figure 49.

Table 33: Percentage Magnetite Entrapped

Formulation code	Magnetite entrapped (%)
F1	21.450
F2	26.813
F3	29.494
P1	21.885
P2	22.522
P3	24.857
Q1	22.522
Q2	25.929
Q3	28.610

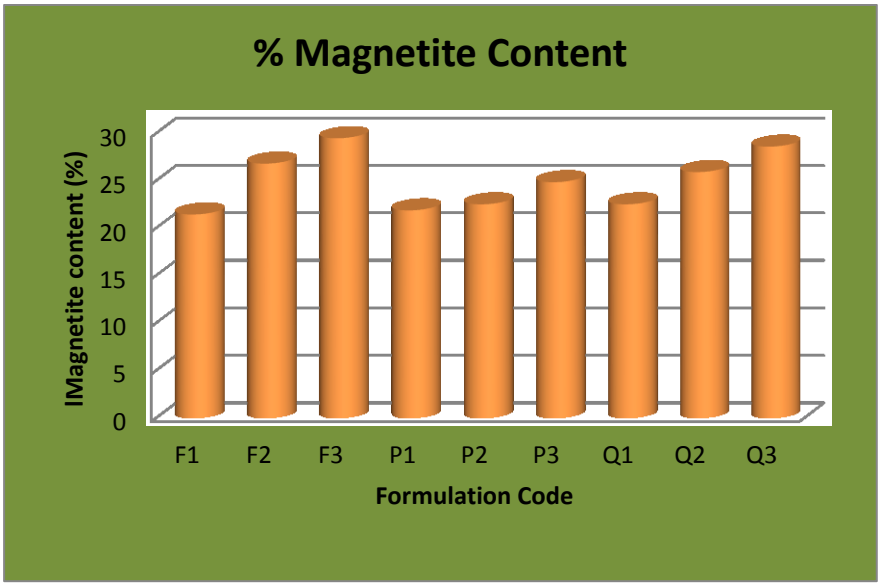


Figure 49: Magnetite content of Formulated Microspheres

The amount of magnetite content per microspheres of weight equivalent to 50mg of magnetite was determined in all formulations. The maximum magnetite content was found to be 29.494% in formulation F3.It was observed that entrapment of magnetite increased with increase in concentration of polymer added in consecutive formulations and the values range from 21.450- 29.494%. The optimum magnetite content is generally between 22-50%.[24,28,82]

9.3.3.2 Magnetic Separation Study[42]

The formulated magnetic microspheres were dispersed in water to study their magnetic separation behavior. Figure shows that the magnetic microspheres rapidly move towards the direction of magnetic field in an aqueous solution under the externally applied magnetic field in 30s. After the ceassation of external magnetic field, the microspheres evenly spread out in water again with bottle shaking.

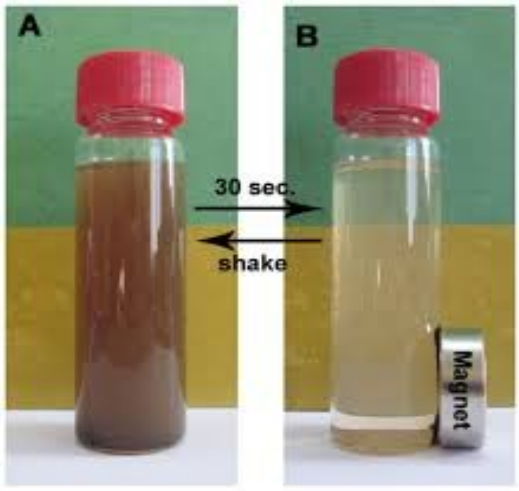


Figure 50: magnetic separation

9.3.3.3 Magnetism Assessment using Vibrating Sample Magnetometer (VSM)[29,42,78]

The magnetization curves of the magnetically responsive microspheres were taken at room temperature using Vibrating Sample Magnetometer (VSM) to study the superpara magnetic behavior by finding the saturation magnetization value.

The prepared mesalamine magnetic microspheres displayed super-paramagnetic property, and the saturation magnetization value was found to be 9.1146emu/g, which is sufficient for magnetic separation from water solution. The saturation magnetization of the microspheres was much lower than that of bulk magnetite (84emu/g) which may be attributed to the incorporation of thick polymer layer on the magnetite particles. As a result, a narrow hysteresis loop was obtained which is shown in the result obtained from VSM. The results are given in table 34 and figure 51.

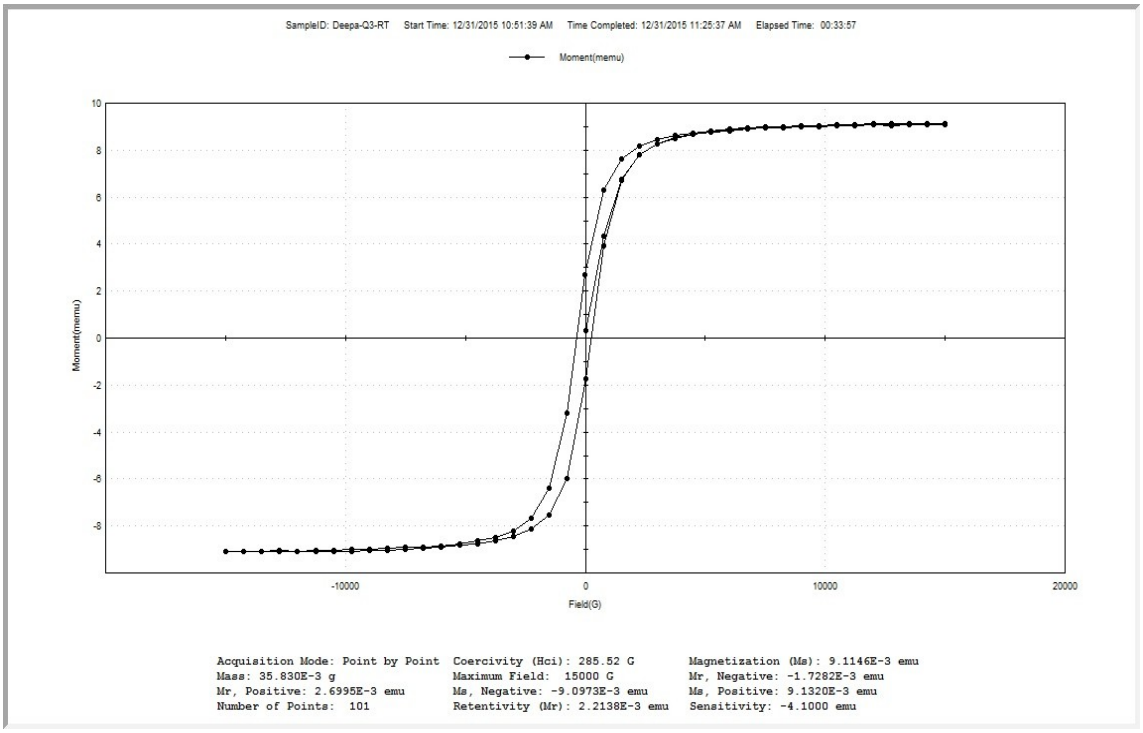


Figure: 51 Magnetization curves obtained by VSM for optimized formulation

Table 34: Parameters analysed by VSM

S. No	Parameter	Result
1	Acquisition Mode	Point by Point
2	Coercivity (Hci)	285.52 G
3	Magnetization (Ms)	9.1146E-3 emu
4	Mass	35.830E-3 g
5	Maximum Field	15000 G
6	Mr, Negative	-1.7282E-3 emu
7	Mr, Positive	2.6995E-3 emu
8	Ms, Negative	-9.0973E-3 emu
9	Ms, Positive	9.1320E-3 emu
10	Number of Points	101
11	Retentivity (Mr)	2.2138E-3 emu
12	Sensitivity	-4.1000 emu

- The aim of the present study was to formulate Magnetically Responsive Mesalamine Microspheres by solvent evaporation method by using biodegradable polymers Chitosan and Pectin and to carry out the various pharmaceutical and magnetic characterizations, to study the effect of polymer type on *in-vitro* drug release and preclinical *in-vitro* screening studies such as *in-vitro* release studies using microflora activated system and *in-vitro* anti-inflammatory activity.

- Chemical compatibility study was performed using FTIR spectroscopy. FTIR spectroscopy studies indicated that the Mesalamine is compatible with polymers. The spectra showed no changes in the major peaks thus confirming no interactions between drug and polymers.

- Calibration curvs of Mesalamine was constructed in Phosphate Buffer Saline pH7.4.

- Magnetite (Fe_3O_4) (used as magnetic carrier) was chemically synthesised using precipitation method.

- In the present study, 9 formulations were prepared in total by using Chitosan and Pectin as polymer in different ratios (1:1, 1:2 and 1:3) of each polymer and combination of two polymers. Also, the effect of polymer type was studied.

- The morphology of optimized formulation was studied by SEM analysis and found that shape of microspheres were discrete, spherical and was satisfactory.

- The mean particle size was found using microscopic analysis, which varied as the drug and polymer ratio changes.

- The drug content for all formulations was found to be in the range of 69.964 to 86.369%w/w. The formulation Q1 had the highest drug content.

- The drug loading was found to be in the range 17.289 – 28.209 %w/w and the data revealed that the drug loading capacity increases with increase in drug:polymer ratio.

- The swelling ability of various microsphere formulations in PBS pH 7.4 was determined and their swelling ratio was found in the range of 0.200 to 0.380. The data revealed that the SR of microspheres increases with decrease in drug content since water molecules cannot acquire much space in MM with higher drug entrapment.

- The *in- vitro* drug release study was carried in phosphate buffer saline pH 7.4 as per the prescribed procedure.

- Among the different formulations, Q1 gave satisfactory results by releasing 100.64 % in 9 hours. The results indicate that the chitosan-pectin microspheres substantially retarded the drug release and showed the best result for the one with higher chitosan content (i.e., Q1 formulation). The inter polymer complex that could be formed between carboxyl groups of pectin and the amino groups of chitosan, may be responsible for such delayed drug release.

- The effect of polymer type on *in-vitro* drug release was compared and from the results, the formulation Q1 has been selected as the optimized formulation substantially retarded the drug release upto 9 hours.

- The *in-vitro* release study of optimized formulation Q1 was applied to various kinetic models to predict the mechanism of drug release. The drug release was found to follow zero order kinetics. In Korsemeyer Peppas equation, the n value was 1.372, indicating anomalous diffusion or non-fickian diffusion, probably Super-Case II transport in which the drug release mechanism may be due to polymer relaxation(erosion) alone.

- Stability studies had been carried out for optimized formulation at a temperature of 40±2°C / 75±5% RH and at ambient room temperature and humidity for a period of 60 days. The results revealed that there is no significant change either in physical appearance or in drug content. Thus the formulation was stable at different conditions of temperature and humidity.

- *in-vitro* drug release study of optimized formulation was carried out in Microflora Activated System containing ß-glucosidase and pectinase enzymes (in an attempt to substitute for rat cecal and colonic enzymes and thereby limiting animal use in the initial screening study). The results show that the polysaccharides undergoes a faster erosion process eventually facilitating faster drug release.

- *in-vitro* percentage inhibition of albumin denaturation method was used to evaluate anti-inflammatory potential for the optimized formulation and the results shows that Q1 formulation has exhibited a satisfactory dose-dependent anti-inflammatory activity for over a period of 9 hrs.

- Magnetite (Fe_3O_4) content in the prepared magnetically responsive microspheres was determined by the conventional titrimetric method using thiosulphate and potassium iodide. The maximum magnetite content was found to be 29.494% in formulation F3.It was observed that entrapment of magnetite increased with increase in concentration of polymer added in consecutive formulations and the values range from 21.450- 29.494%. The optimum magnetite content is generally between 22-50%.

- The magnetization curves of optimized formulation Q1 was taken using Vibrating Sample Magnetometer (VSM) to study the super-paramagnetic behaviour. The result shows that the saturation magnetization value was found to be 9.1146emu/g, which is sufficient for magnetic separation from water solution. A narrow hysteresis loop was obtained from VSM which confirms that the formulation displayed a satisfactory super-paramagnetic property.

- It can be concluded that the Magnetically Responsive Mesalamine Microspheres offer a localized drug delivery only at the target site by the combined effect of **physical approach** (utilizing the principle of magnetic targeting with an intention to produce a depot near the target organ) and **biochemical approach (**using biodegradable polymers chitosan and pectin for drug release in a controlled manner). By producing a depot near the target organ, unwanted distribution of drug to non target organ can be avoided.

- The results of the study revealed Magnetically Responsive Mesalamine Microspheres is an effective strategy for localized drug delivery only at the target site for the treatment of Irritable Bowel Diseases and thereby minimizing the dose and drug induced toxicity.

FUTURE SCOPE

- *in-vitro* screening using cell culture models
- *in- vivo* screening using animal models (using gamma scintillography).
- Pharmacokinetic and toxicity study

The adoption of magnetic particles for targeted delivery is minimal and most of the work is in the basic research phase. Hence their potential is yet to be realized fully. The future holds great promise for its systematic investigation and exploitation.

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